Cost of Predator-Induced Plasticity and Cost of Responding to Predators in Tadpoles

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CONTENTS

GENERAL INTRODUCTION

The world is heterogeneous in time and space. To cope with this variation is an ongoing and fascinating story of evolution. Organisms should evolve adaptive phenotypes to match their environment and track environmental change (Roff 1992, Stearns 1992). However, most environmental variation occurs at a time scale too fast to be tracked by evolutionary change. A solution to this problem is the evolution of environmentally induced phenotypes, called phenotypic plasticity. Phenotypic plasticity is the ability of a single genotype to express various phenotypes in different environments (Bradshaw 1965, Stearns 1989, West-Eberhard 1989, Schlichting and Pigliucci 1998, Tollrian and Harvell 1999, Pigliucci 2001). Given that environmental variability is ubiquitous, phenotypic plasticity is also almost ubiquitous. Phenotypic plasticity raises questions about how phenotypes are formed on a genetic and developmental level, about ecological consequences of phenotypic plasticity for the life of individuals, as well as the structure and dynamics of populations and communities. It is thus a link -or even *the* link- between genetics, development, physiology, ecology and evolution, and it has been viewed as the unifying concept in biology (Schlichting and Pigliucci 1998).

Evolution of phenotypic plasticity

In an ideal world, an individual would alter its phenotype to always match the optimum. In the real world this is not always true. We still see constitutive (fixed) phenotypes that are not adaptive to all environments and even plastic genotypes do not match their optimum. So, where does the discrepancy between the ideal and the real world come from? Various models predict that phenotypic plasticity should evolve in heterogeneous, stochastic environments, in which reliable environmental cues exist that indicate the current or future environmental conditions (Via and Lande 1985, van Tienderen 1991, Moran 1992); otherwise natural selection favors fixed phenotypes. Anti-predator defenses, for example, are expected to be inducible only if they are costly to produce or maintain (Van Buskirk 2000). If the response is inducible, individuals can avoid paying the costs when predators are absent. Such costs associated with the expression of traits in different environments have been measured in many plants and animals (Stemberger 1988, West-Eberhard 1989, Harvell 1992, Lampert et al. 1994, Pettersson and Brönmark 1997, Harvell 1998, Schlichting and Pigliucci 1998, Tollrian and Harvell 1999, Van Buskirk 2000, Pigliucci 2001, Van Buskirk and Saxer 2001, Agrawal et al. 2002, Tiffin 2002).

Another sort of cost, the cost of being plastic, occurs regardless of the trait value expressed. Costs of plasticity limit the evolution of phenotypic plasticity and therefore are one of the key barriers preventing genotypes from adjusting their phenotypes at will to heterogeneous environments. Various types of costs of plasticity have been delineated, such as maintenance costs of the sensory and regulatory mechanism of plasticity, production costs of plastic genotypes that exceed those paid by fixed genotypes, information acquisition costs, e.g. sampling information under risky situations, costs of developmental instability, and genetic costs, such as negative pleiotropic effects of plasticity genes on traits other than the plastic ones, and costs of epistasis where regulatory loci producing plasticity may modify the expression of other genes (DeWitt et al. 1998). Costs of plasticity may thus explain why in the real world plastic phenotypes do not match the optimum. Therefore, plastic genotypes have lower fitness than non-plastic genotypes for a given trait value, yet this difference must be low to allow the evolution of plasticity (van Tienderen 1991). It is not surprising that costs of plasticity have attracted substantial attention, though empirical evidence for them is ambiguous (DeWitt 1998, Scheiner and Berrigan 1998, Tucic et al. 1998, Donohue et al. 2000, Dorn et al. 2000, van Kleunen et al. 2000, Smekens and van Tienderen 2001, Tucic and Stojkovic 2001, Agrawal et al. 2002, Relyea 2002, Steinger et al. 2003, Merilä et al. 2004, Stinchcombe et al. 2004).

Studying phenotypic-plasticity

When studying phenotypic plasticity, we should know the inducing environmental factor and how natural selection acts on trait variation that is the result of phenotypic plasticity. This is the case for predator-induced or herbivore-induced defenses, which thus became model-systems for the study of phenotypic plasticity. The benefits of defended genotypes are strong and relatively easy to measure (Schlichting and Pigliucci 1998, Tollrian and Harvell 1999, Pigliucci 2001), and environmental variation in predation or herbivory is easily manipulated by either excluding or including predators or herbivores, or mimicked by adding caged (thus non-lethal) predators, or mechanically damaging plants. Predators or herbivores release chemical cues such as kairomones and pheromones while digesting, and prey or plants release alarm cues when injured. These cues induce physiological, morphological, life-historical or behavioral defenses in the injured and/or other individuals. The consequences of predator-prey interactions are mostly clear cut: live or die; for plants the consequence of herbivory is often not as clear cut but still well correlated with fitness. Hence, the beneficial nature of phenotypic plasticity can easily be established.

Being able to exclude or at least account for genetic variation among individuals is another criterion for model systems to study plasticity. Excluding genetic variation by using individuals of a single genotype ascertains that the differences in trait expression between environments indeed arise from induced plastic responses. For practical purposes, "genotypes" can be clonal lineages or full-sib families. Variation in plasticity between genotypes is desirable for the study of plasticity.

Tadpoles as model system for plasticity

One of these model systems for the study of phenotypic plasticity is tadpoles exposed to predation by dragonfly larvae (Werner and Anholt 1993, Relyea 2002, Van Buskirk 2002a, Merilä et al. 2004). Tadpoles have evolved multiple complex anti-predator defenses, many of which are inducible, such as behavioral, morphological and life-historical responses (Werner and Anholt 1993, Smith and Van Buskirk 1995). The induction of responses is triggered by kairomones and alarm cues released by predators or injured prey and can easily be manipulated in a non-lethal way (Wisenden 2000, Schoeppner and Relyea 2005). In response to predator-released chemical cues, tadpoles respond adaptively by reducing their swimming and feeding activity, and by increasing the size of their tail fin and muscle. But they also have to pay compensatory costs for these adaptive responses, resulting in "maladaptive" responses such as reduced growth and prolonged time to metamorphosis. Reduced feeding and increased investment in tail depth are known to reduce predation rate (Van Buskirk and McCollum 2000a, b), whereas reduced size (growth) and the prolonged time being exposed to predators (delayed metamorphosis) increase the predation probability (Arendt 1997). Tadpoles also became a model system because within a single clutch there are thousands of full sibs; once hatched these tadpoles are easy to raise in different environments. In my dissertation I used this model system to carry out a series of experiments to explore the evolution of phenotypic plasticity with a special focus on costs that enforce and limit the evolution of plasticity, and some excursions into the ecology of plasticity and its consequences.

Gaps in the study of phenotypic plasticity

We have a fairly good theoretical understanding of the evolution of phenotypic plasticity, i.e. how trait expression costs enforce the evolution of plasticity while costs of plasticity restrict its extent (Via and Lande 1985, van Tienderen 1991, Gomulkiewicz and Kirkpatrick 1992, DeWitt et al. 1998, Scheiner and Berrigan 1998, Sultan and Spencer 2002, Ernande and

Dieckmann 2004). Our mechanistic understanding is weak, as is the empirical evidence for many of the predicted effects associated with phenotypic variation.

Despite substantial effort to detect costs of plasticity, no study has yet found convincing evidence for such costs. Apart from the theoretical prediction that these costs should be small, we have no understanding how large they in fact are. Empirical studies have not quantified these costs and surprisingly no power analyses for any empirical study exist that predict how large the costs would have to be for detection. In chapter one, I discuss the question why there are so many ambiguous results for costs of plasticity, and I present convincing evidence for costs of plasticity and quantify these costs for the first time.

Although many studies deal with predator-induced responses in tadpoles, the costs associated with these responses and the linking mechanisms behind these responses are not well understood. Specifically, potential co-evolution of defense traits and non-linear response patterns along environmental gradients have not been studied. This applies not only to tadpoles but also to other model systems used to study plasticity (Angilletta et al. 2003). Time and resource allocation trade-offs should be the driving force for the co-evolution and co-specialization of multiple traits. So far only separate models for time and resource allocation trade-offs existed. I filled this gap by integrating both time and resource allocation trade-offs within a single model to predict investment in behavioral and morphological defenses under various environmental conditions (chapter two). Only a model that incorporates both trade-offs predicts co-evolution and co-specialization of multiple defense traits, dependent on environmental conditions. The model also predicts non-linear response patterns of investment in defense that have not been predicted before in animal systems. In chapter three, some of these predictions are tested and confirmed, such as peak investment in behavioral defense at intermediate resource availability, while other predicted patterns of various models do not fit the empirical data.

Finally, physiological responses, which are the main focus of studies on inducible defenses in plants, are mostly ignored or not known in animal systems for phenotypic plasticity. To meet this shortcoming, I explored such physiological and internal morphological responses to predation risk in tadpoles in chapter four. I show how costs of avoiding predators can be minimized by modifying physiological mechanisms in response to predators. More physiological mechanisms accompanying behavioral and morphological plastic responses await to be explored.

Although I made good progress in the study of phenotypic plasticity, many open questions remain. Among the most obvious gaps in my opinion is the lack of information about the genetic

background of plasticity, such as genetic linkage, co-evolution and co-speciation of traits. Another wide open field is the correlation between various plasticities across various heterogeneous environments, because plasticity was mostly assessed for only one kind of habitat heterogeneity.

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SUMMARY

Adaptation to variable environment is one of the most challenging, but also most essential problems organisms face in the course of life. Phenotypic plasticity, the ability of a single genotype to express various phenotypes, is one of the most beneficial solutions to this problem, as shown by its wide occurrence. Plasticity should evolve in heterogeneous, stochastic environments. Trait expression costs or costs of responses are also necessary to explain the evolution of inducible phenotypes. A fixed phenotype has to pay costs to build a trait irrespective of the experienced environment, while a plastic phenotype can save some costs in environments where the cost to build the trait outweighs the adaptive advantage. Other than trait expression costs, costs of being plastic occur regardless of the trait value expressed. Such plasticity costs limit the evolution of phenotypic plasticity and they are the key barrier for genotypes to shape their phenotypes at will. Plasticity costs are recognized when plastic genotypes have relatively low fitness regardless of the trait value expressed, compared to fixed genotypes. In this dissertation, I address questions about the evolution of phenotypic plasticity by exploring both types of costs, the costs of responses and the cost of plasticity. The first enforce and the latter limit the evolution of plasticity. To assess these costs, I used a predator-prey model system with *Rana temporaria* and *Rana lessonae* tadpoles and explored their responses to predatory *Aeshna* dragonfly larvae.

In **chapter one**, we study why no convincing evidence for plasticity costs was revealed in a number of previous studies. Impediments to find cost of plasticity in these empirical studies are most likely due to methodological problems. Problems include, (1) benign laboratory conditions under which plasticity costs might be small, (2) weak correlations of fitness measures with lifetime fitness, and (3) separate analysis for single traits that unlikely reflect whole-organism plasticity. We report a series of experiments to explore costs of predator-induced plasticity in frog tadpoles in which we attempted to avoid these methodological problems. We assessed costs in benign and stressful conditions, using measures of fitness differing in sensitivity, and assessed correlations in plasticities between multiple traits to evaluate the likelihood for an aggregate measure of overall plasticity. We report good evidence for cost of plasticity, but only when we apply a sensitive fitness measure. Hence, costs of plasticity are small and therefore hard to detect. Our results show no evidence for any differences in costs between benign and stressful environments and an aggregate measure of overall plasticity does not exist.

The following chapters study costs of responses to predators. In **chapter two** we develop a mathematical model that comprises time and resource allocation trade-offs for multiple defense traits. Time allocation trade-offs partition time, e.g. between foraging effort to acquire resources and behavioral defense. Resource allocation trade-offs partition the acquired resources between multiple traits, such as growth or defense. In the model, we maximize survival during ontogeny by optimizing investment in behavioral and morphological defense for different environments. We compare the results of the integrated model with simplified versions that optimize only a single defense trait. We show that behavioral and morphological defense compensate for and augment each other dependent on predator densities and effectiveness of the defense mechanisms. The response patterns to predator density and resource availability are more pronounced in the simplified settings compared to the integrated optimization. This implies that the evolution of integrated traits is favored and simplification overestimates the effects on population and community dynamics. When incorporating time constraints, the model shows a peak investment in optimal morphological and behavioral defense at intermediate resource availability. This does not occur in the absence of time constraints.

In **chapter three** I explore some of the predictions of the model described in chapter two, by studying the integration of behavioral and morphological defensive responses and their costs. The expression of defensive traits is determined by the trade-off between the effectiveness of defense in each trait and fitness costs of building the trait. The costs in responding to predators caused by defensive traits have to be compensated by fitness traits and should, thus, be measurable by reduced expression in fitness traits. If defensive traits vary in their costliness, shifts in trait expressions should occur with varying resource availability. Models based on time and resource allocation trade-offs, as described in the previous chapter, predict different outcomes along a resource availability gradient. For behavioral traits and costly defensive traits, an increased investment in defense is predicted by acquisition and basic allocation models. Alternative models, such as the growth-differentiation-balance-hypothesis or our model described in chapter 2, predict a peak in investment in defense at intermediate resource availability. I estimated the costs of six fitness and/or defensive traits by exposing tadpoles to a resource availability gradient and measured the investment in defense by assessing the plasticity to predator exposure. The results of the experiment show that the costs of responding to predators in defensive traits are not primarily energetic and the investment in defense did not follow the predictions of the considered models. Costs of responses to predators, caused by increased tail

depth and reduced activity, were compensated by a reduced developmental rate and a reduced survival. At low resource availability the response costs were not compensated by other fitness traits and the consequence was a reduced survival, while at high resource availability the compensation shifted towards an increasingly reduced developmental rate.

In the previous two chapters I focused on behavioral and external morphological responses and their costs. In **chapter four**, I study predator-induced physiological and internal morphological responses, which are, in contrast to a variety of responses related to behavior and morphology, not well known. Behavioral models assume that the costs of morphological and behavioral defenses stem from reduced feeding and are expressed in reduced growth. Physiological and digestive mechanism should link the induced behavioral and external morphological responses. I tested these model assumptions, by experimentally investigating the effects of exposure to predators on ingestion rate, intestinal morphology, and assimilation rate in tadpoles. Predator-exposed tadpoles showed shorter guts. Despite a reduction in time spent feeding, predator-exposed individuals ingested the same amount of food and assimilated the food at a higher rate, but did not grow faster. Models predicting increased ingestion with increased feeding to maximize growth are misleading. Instead, optimization models that allow intrinsic costs of ingestion and digestion are supported by my results. Costs of avoiding predators, e.g. a reduced feeding rate, can be minimized by modifying ingestion and assimilation rate in response to predators. However complete physiological compensation for the costs of avoiding predators is apparently not possible; and other physiological costs are required to explain why individuals exposed to predation pressure usually grow slowly.

ZUSAMMENFASSUNG

Die Anpassung an variable Umwelten ist eine der schwersten, aber gleichzeitig auch verbreitetsten Herausforderungen, der sich Organismen im Laufe ihres Lebens stellen müssen. Phänotypische Plastizität, also die Fähigkeit eines einzigen Genotyps, mehrere Phänotypen auszubilden, ist eine der elegantesten Antworten auf diese Herausforderung. Dies wird nicht zuletzt durch die weite Verbreitung von Phänotypischer Plastizität unterstrichen. Evolutive Modelle sagen vorher, dass sich Plastizität in heterogenen stochastischen Umwelten entwickelt.

Um die Evolution solcher induzierbarer Phänotypen zu erklären, müssen die Kosten, die bei der Ausbildung von Merkmalen entstehen, mit betrachtet werden. Ein fixer Phänotyp trägt, unabhängig von den unmittelbaren Lebensbedingungen seiner Umwelt, immer die gleichen Kosten für die Ausprägung eines (fixen) Merkmals, während ein plastischer Phänotyp Kosten sparen kann, wenn die Vorteile der plastischen Merkmalsausprägung in dieser Umwelt grösser sind als die Kosten ein (plastisches) Merkmal auszuprägen. Zusätzlich zu den Kosten der Merkmalsausprägung entstehen weitere Kosten durch die Phänotypische Plastizität, unabhängig von der Merkmalsausprägung. Solche Kosten schränken die Evolution von Plastizität ein und sind daher eines der Haupthindernisse für Genotypen, beliebige Phänotypen auszubilden. Kosten einer Phänotypischen Plastizität können anhand einer verringerten Fitness (die plastische Genotypen, im Gegensatz zu fixen Genotypen, unabhängig von ihrer Merkmalsausprägung aufweisen) aufgezeigt werden.

In dieser Doktorarbeit beschäftige ich mich mit Fragen der Evolution von Phänotypischer Plastizität, indem ich beide Arten von Kosten, die Kosten der Merkmalsausprägung und die Kosten von Plastizität, betrachte. Die Kosten der Merkmalsausprägung fördern die Evolution von Plastizität, während Kosten von Plastizität selbige einschränken. Ich bestimme diese Kosten in einem Räuber-Beute-Modelsystem: Dazu betrachtete ich die Reaktionen von Kaulquappen der zwei Froscharten *Rana temporaria* und *Rana lessonae* auf räuberische *Aeshna*-Libellenlarven.

Im **ersten Kapitel** beschäftige ich mich mit der Frage, weshalb in mehreren vorangegangenen Studien kein überzeugender Beweis für die Kosten der Plastizität vorgelegt werden konnte. Vermutlich lag die Problematik, solche Kosten zu ermitteln, in der Methodik. Folgende Schwierigkeiten treten auf: (1) vorteilhafte Laborbedingungen, unter denen die Kosten der Plastizität gering sein könnten, (2) schwache Korrelationen der verwendeten Fitnessmasse mit der Gesamtfitness eines Individuums (lifetime fitness), und (3) separate Analysen für einzelne Merkmale, welche nur mit geringer Wahrscheinlichkeit die gesamtheitliche Plastizität

eines Organismus widerspiegeln. Ich beschreibe eine Reihe von Experimenten, die Kosten von räuberinduzierter Plastizität bei Froschkaulquappen belegen, wobei ich versucht habe, die oben aufgezählten methodischen Probleme zu vermeiden. Ich ermittelte die Kosten unter guten und von Stress geprägten Umweltbedingungen, verwendete unterschiedlich sensitive Fitnessmasse und betrachtete Korrelationen der Plastizität mehrerer Merkmale, um die Wahrscheinlichkeit eines universalen Masses für gesamtheitliche Plastizität abzuschätzen. Eindeutige Kosten der Plastizität konnten aufgezeigt werden, allerdings unter dem Vorbehalt, dass ein sensitives Fitnessmass verwendet wird. Dies deutet darauf hin, dass die Kosten der Plastizität klein sind, und erklärt, weshalb ihre Existenz schwierig nachzuweisen ist. Meine Ergebnisse geben keine Hinweise auf unterschiedliche Kosten von guten und von Stress geprägten Umweltbedingungen. Ein universales Mass für gesamtheitliche Plastizität scheint nicht zu existieren.

In den folgenden drei Kapiteln werden die Kosten behandelt, die bei der Reaktion auf Räuber entstehen. In **Kapitel zwei** entwickele ich ein mathematisches Modell, das Zeit- und Ressourcen-Zuordnungs-Abwägungen (time und resource allocation trade-offs) für mehrere Verteidigungsmechanismen gleichzeitig berücksichtigt. Zeit-Zuordnungs-Abwägungen teilen die verfügbare Zeit beispielsweise zwischen Futtersuche und -aufnahme und Verteidigungsverhalten auf. Ressourcen-Zuordnungs-Abwägungen verteilen die aufgenommenen Futtermengen zwischen mehreren Merkmalen, wie zum Beispiel Wachstum oder Verteidigung. Mein integriertes Modell, das mehrere Verteidigungsmechanismen gleichzeitig optimiert, wird mit vereinfachten Modelleinstellungen, welche nur einen Verteidigungsmechanismus optimieren, verglichen. Das Modell basiert auf der Maximierung der Überlebenswahrscheinlichkeit während der Ontogenese, indem es den Aufwand für Verteidigungsverhalten und morphologische Verteidigungsmechanismen in unterschiedlichen Umwelten optimiert. Die Ergebnisse zeigen, dass Verteidigungsverhalten und morphologische Verteidigungsmechanismen sich je nach Räuberdichte und Effizienz des Verteidigungsmechanismus entweder ergänzen oder gegenseitig ersetzen. Die Reaktion auf Räuberdichte und Futterverfügbarkeit ist im vereinfachten Modell ausgeprägter als im integrierten. Hieraus lässt sich schliessen, dass die Evolution von integrierten Merkmalen und Mechanismen bevorzugt wird und dass es durch Vereinfachungen zur Überschätzung der Effekte von Reaktionen auf Räuberdruck in Bezug auf die Dynamik von Populationen und Lebensgemeinschaften kommt. Wenn zeitliche Limitierungen im Modell mit eingegliedert werden (z.B. Ende der Wachstumssaison), wird der höchste Aufwand für

Verteidigungsverhalten und morphologische Verteidigung bei mittlerer Futterverfügbarkeit beobachtet. Ein solcher Effekt tritt in dem zeitlich unlimitierten Modell nicht auf.

Im **dritten Kapitel** betrachte ich einige der Vorhersagen, welche aus dem in Kapitel zwei beschriebenen Modell hervorgehen. Dazu betrachte ich das Zusammenwirken von Verhaltensverteidigungs- und morphologischen Verteidigungsreaktionen sowie deren Kosten. Die Ausprägung von Verteidigungsmerkmalen wird durch das Verhältnis der Effektivität eines jeden Merkmals zu dessen Fitnesskosten der Merkmalsausbildung bestimmt. Es wird angenommen, dass die Kosten, auf Räuber zu reagieren, durch die Ausbildung von Verteidigungsmerkmalen hervorgerufen werden. Diese Kosten müssen durch Fitnessmerkmale kompensiert werden und sollten daher durch eine reduzierte Ausprägung dieser Merkmale messbar sein. Wenn Verteidigungsmerkmale unterschiedlich hohe Kosten verursachen, sollte sich der Grad der jeweiligen Merkmalsausprägung bei unterschiedlicher Futterverfügbarkeit unterscheiden. Modelle, welche auf Zeit- und Ressourcen-Zuordnungs-Abwägungen beruhen, wie jenes aus dem vorhergehenden Kapitel, machen bezüglich der Reaktionen entlang eines Futterverfügbarkeitsgradienten unterschiedliche Vorhersagen. Für Verteidigungsverhalten und kostenintensive Verteidigungsmerkmale wird von einfachen Zeit- und Ressourcen-Zuordnungs-Abwägungs-Modellen ein zunehmender Verteidigungsaufwand mit zunehmender Futterverfügbarkeit vorhergesagt. Alternative Modelle, wie die Wachstums-Differenzierungs-Ausgleichs-Hypothese *(Growth-differentiation-balance-hypothesis)* oder auch unser integriertes Modell aus Kapitel zwei, sagen bei mittlerer Futterverfügbarkeit einen maximalen Aufwand für Verteidigung voraus. Um diese Voraussagen zu testen, schätzte ich die Kosten von sechs Fitness- und/oder Verteidigungsmerkmalen ab. Dazu setzte ich Kaulquappen einem Futterverfügbarkeitsgradienten aus. Den Aufwand für Verteidigung mass ich durch die Aufnahme der räuberinduzierten Plastizität. Die Ergebnisse des Experiments zeigen, dass die Kosten, welche durch die Ausprägung von Verteidigungsmerkmalen auf Räuber hervorgerufen werden, nicht primär energetischer Natur sind. Gleichzeitig stimmte der Aufwand für Verteidigung nur teilweise mit den Modellvorhersagen überein. Räuberinduzierte Kosten, welche durch höhere Schwanzflossen und geringere Aktivität entstanden, wurden je nach Futterverfügbarkeit durch eine geringere Entwicklungsrate und geringere Überlebenswahrscheinlichkeit ausgeglichen. Bei geringer Futterverfügbarkeit wurden die Reaktionskosten nicht von anderen Fitnessmerkmalen aufgefangen; als Konsequenz folgte eine

geringere Überlebenswahrscheinlichkeit. Hingegen wurden bei hoher Futterverfügbarkeit die Reaktionskosten durch eine reduzierte Entwicklungsrate kompensiert.

In den vorangehenden zwei Kapiteln habe ich den Schwerpunkt auf Verteidigungsverhalten und äussere morphologische Reaktionen und deren Kosten gelegt. Im **vierten Kapitel** untersuche ich räuberinduzierte physiologische und innere morphologische Verteidigungsantworten, über die, im Gegensatz zu einer Reihe von Verhaltens- und morphologischen Antworten, wenig bekannt ist. Vorhersagen von Verhaltensmodellen sind: Die Kosten des Verteidigungsverhaltens und der morphologischen Verteidigung stammen von einer reduzierten Fressaktivität und drücken sich in einer verringerten Wachstumsrate aus. Physiologische und Verdauungsmechanismen sollten die induzierten Verhaltens- und die externen morphologischen Antworten miteinander koppeln. Ich testete diese Modellannahmen, indem ich die Effekte von Räuberdruck auf die Futteraufnahmerate, die innere Morphologie und die Assimilationsrate bei Kaulquappen untersuchte. Kaulquappen, die Räubern ausgesetzt waren, entwickelten kürzere Därme. Obwohl diese Kaulquappen weniger Zeit zum Fressen aufwendeten, nahmen sie die gleiche Menge Futter auf und assimilierten das Futter zu einem höheren Grad, wuchsen jedoch nicht schneller. Modelle, die aufgrund von längeren Frasszeiten eine erhöhte Futteraufnahme und daher ein stärkeres Wachstum vorhersagen, sind irreführend. Stattdessen werden meine Daten von Optimierungs-Modellen unterstützt, welche inhärente Kosten von Futteraufnahme und Verdauung berücksichtigen. Kosten, die bei der Vermeidung von Räubern entstehen, wie z. B. eine herabgesetzte Aktivität, können verringert werden, indem die Futteraufnahme und Assimilationsrate bei Räuberdruck verändert wird. Jedoch kann kein vollständiger physiologischer Ausgleich für Kosten, die durch die Vermeidung von Prädation entstehen, vorgenommen werden. Andere physiologische Kosten sind zur Erklärung nötig, weshalb Individuen, die Räubern ausgesetzt sind, normalerweise langsamer wachsen.

CHAPTER 1

COSTS OF PHENOTYPIC PLASTICITY: WHY SO MANY AMBIGUOUS RESULTS?

Ulrich K. Steiner and Josh Van Buskirk

Abstract

Costs of plasticity play a central role for the evolution of phenotypic plasticity, because such costs are the key barrier for genotypes to shape their phenotypes at will to heterogeneous environments. Plasticity costs are recognized when plastic genotypes have relatively low fitness regardless of the trait value expressed. No convincing evidence for plasticity costs was revealed in a number of previous studies, most likely due to methodological problems. Problems include, (1) benign laboratory conditions under which plasticity cost might be small, (2) weak correlations of fitness measures with lifetime fitness, and (3) separate analysis for single traits that unlikely reflect whole-organism plasticity. We report a series of experiments to explore costs of predator-induced plasticity in frog tadpoles in which we attempted to avoid these methodological problems, by assessing costs in benign and stressful conditions, using different sensitive measures of fitness, and assessing correlations in plasticities between traits to evaluate the likelihood for an aggregate measure of overall plasticity. We report good evidence for cost of plasticity, but only when we apply a sensitive fitness measure. Hence, costs of plasticity are small and therefore hard to detect. Our results showed no evidence for any differences in costs between benign and stressful environments and an aggregate measure of overall plasticity does not exist.

Introduction

Costs of plasticity play a central role in the theory of phenotypic evolution. These costs are recognized when plastic genotypes have relatively low fitness regardless of the trait value expressed. Models that include no fitness costs of expressing phenotypic plasticity suggest that heterogeneous environments should often favor the evolution of plasticity (Via and Lande 1985). In this case the optimal genotype is that which expresses trait values conferring the highest fitness in all environments that are encountered. The cost of plasticity can be an important impediment to this outcome (van Tienderen 1991). Thus, plasticity costs have attracted attention because they seem to be a barrier preventing organisms from shaping themselves at will to match their environments (DeWitt et al. 1998).

The cost of plasticity is estimated by regressing fitness against phenotypic plasticity after accounting for natural selection acting directly on the phenotype (van Tienderen 1991). Many studies have applied this method in diverse organisms, but none has reported convincing evidence for plasticity costs. In a survey of 13 publications, we found 315 tests each comparing a component of fitness with plasticity in a particular trait (DeWitt 1998, Scheiner and Berrigan 1998, Tucic et al. 1998, Donohue et al. 2000, Dorn et al. 2000, van Kleunen et al. 2000, Smekens and van Tienderen 2001, Tucic and Stojkovic 2001, Agrawal et al. 2002, Relyea 2002, Steinger et al. 2003, Merilä et al. 2004, Stinchcombe et al. 2004). Costs and benefits of plasticity were about equally common in this sample. There were 157 negative relationships, as expected if plasticity is costly (20 were significant), and 146 positive relationships (14 significant). Twelve tests showed no tendency. This suggests that costs of plasticity must be very small, or are being measured in the wrong way.

We suspect that the ambiguity arises partly from methodological problems with the empirical studies. For example, most experiments to date have been conducted under relatively benign conditions, often in the laboratory. The cost of plasticity may be higher in stressful environments (Steinger et al. 2003), because when conditions are poor there is less opportunity to compensate for investment in the capacity to show plasticity. Such allocation-based tradeoffs are probably weak in the case of plasticity costs, but nevertheless the benign conditions in most experiments may reduce the likelihood of detecting fitness variation and costs. This problem could be solved by performing tests under more stressful conditions.

A second problem with existing tests is that they usually monitor indirect correlates of fitness. Performance components that span just part of the life cycle represent an incomplete

picture of true fitness, and therefore introduce error into the analysis (Endler 1986). This reduces the likelihood of detecting fitness costs that may be small. Ideally, this problem would be solved by estimating life table parameters directly, but this is infeasible for most species. At the very least, a test for costs of plasticity requires measures of fitness components that are well-correlated with lifetime fitness.

A third problem with all published studies except one (Donohue et al. 2000) is that they subject each plastic trait to a separate analysis. These studies begin by estimating selection acting on one trait, to the exclusion of all others, and then ask whether remaining variation in fitness is associated with plasticity in that single trait. The univariate analysis is an inadequate description of natural selection on the phenotype if multiple traits jointly influence fitness, which is typical (Schluter and Nychka 1994). Also, the method has a poor chance of detecting costs if they arise from plasticity in many different traits, which seems likely. This would be less problematic if plasticity in all traits is positively correlated, so that results for individual traits accurately reflect costs of whole-organism plasticity. One solution here is to test for correlations among traits in phenotypic plasticity; if relatively plastic genotypes have high plasticity for all traits, then analysis should be performed on a single aggregate measure of whole-organism plasticity.

We have attempted to avoid these methodological problems in our study of the cost of predator-induced plasticity in frog tadpoles. First, we checked if an aggregate measure of overall plasticity exists, which could be used to test for potential plasticity costs. Atomizing the phenotype into distinct traits will be necessary if plasticities in different kinds of traits are negatively correlated. Second, we compared two kinds of fitness components to determine if costs of plasticity are more conspicuous in more sensitive (less variable) measures of fitness. Finally, we asked if estimates of plasticity costs vary among environments, with the expectation that costs may be larger or more easily detected in stressful environments.

Methods

Our study consisted of two experiments, each involving a set of 40 full-sib families (sibships). The first, which we call the plasticity experiment, provided data on plastic responses to two environments, along with a measure of fitness in both environments. The second experiment, called the competition experiment, provided an independent measure of fitness by subjecting each sibship to competition with other sibships at two different densities.

We collected 40 clutches of *Rana temporaria* eggs (full-sib families) in March 2003 from

eight populations (1.8 km to 45 km apart) in Kantons Thurgau and Zürich, Switzerland. Clutches from different populations were used to maximize genetic variability in plasticity. Both experiments reared the tadpoles in outdoor artificial ponds in a field at the University of Zürich. The ponds were plastic tubs (0.28-m^2, 80 L), containing 60g of dried leaf litter and a diverse community of zooplankton and algae. We stocked the ponds with tadpoles at Gosner (1960) stage 26 on 11 April 2003 (plasticity experiment) and 12 April (competition experiment). Tadpoles were 13-14 days old when the experiments began.

Plasticity experiment

We measured predator-induced plasticity in eight traits by rearing each of the 40 sibships in the presence and absence of dragonfly larvae, with two replicates in randomized blocks (total of 160 ponds). Every pond received 12 tadpoles (43 tadpoles/m^2) originating from a single sibship. The ponds were outfitted with a floating cage (~1 L volume), which either contained one final instar dragonfly (*Aeshna cyanea*) or was left empty, depending on the treatment. The dragonflies were fed 300 mg of *R. temporaria* tadpoles three times a week, and the cages within the predator treatment were rotated on feeding days to equalize any differences among individual *Aeshna*. Cages in the predator-free ponds were also rotated to control for effects of disturbance. Four tubs were accidentally lost before the end of the experiment.

We measured phenotypic plasticity in early May. Morphological measurements came from eight randomly selected tadpoles in each pond, which were weighed and photographed in side and bottom view on 7-9 May. We used image analysis software to measure the maximum depth and length of the tail, the width, depth and length of the head, and tadpole size. The tail and head measures were corrected for variation in tadpole size by first regressing against body size and the square of size. Body size was the centroid size calculated from 21 landmarks positioned in three-dimensional space (Bookstein 1991). The behavioral data came from six instantaneous samples of each tub over a 4.5-hour period on 6 May, a sunny and warm day. We recorded the number of visible tadpoles that were active and inactive; non-visible tadpoles were counted as hiding.

Plasticity in each of the eight traits was defined as the difference in sibship means between the predator-naïve and predator-exposed tadpoles. The plasticity experiment also provided an estimate of tadpole fitness (mass at 5 weeks of age) in two environments with and without caged predators. Body mass is a good measure of fitness for tadpoles, because it is negatively correlated with mortality from predation and positively related to size and survival

during the adult stage (Smith 1987, Scott 1994, Altwegg and Reyer 2003).

Competition experiment

We used the same 40 sibships in a competition experiment to measure performance under stressful conditions, and to apply a more sensitive measure of fitness related to competitive ability. Each sibship was competed against two other sibships from different populations, at low and high density (8 tadpoles/pond or 24/pond; 29 tadpoles/m^2 or 86/m^2). Survivors were collected when they reached metamorphosis (stage 42), weighed at tail resorption, and preserved in 70% ethanol for molecular analysis.

We assigned metamorphs to their sibship of origin using microsatellite markers (Rtempu7 and Rtempu8 from Rowe and Beebee (2001); Rt2Ca36 from Garner, unpublished) and following standard methods of DNA extraction, PCR amplification, and gel electrophoresis (Garner et al. 2000). Only the first four metamorphs were screened in the high-density treatment. Six of 160 metamorphs in the high-density treatment could not be assigned with certainty and were excluded from analysis.

Statistical analyses

We began with a multivariate ANOVA testing effects of sibship and predator treatment in the plasticity experiment on three head and two tail traits, two behavioral traits, and body size. The goal was to verify that sibships differed in plasticity. Sibship was a random effect tested over the predator-by-sibship effect. Behavioral traits were arcsin-square root transformed.

The next step was to determine whether an aggregate measure could be used to summarize variation among sibships in phenotypic plasticity. This was done with a Principal Component Analysis on the correlation matrix of proportional plasticities in the eight focal traits. Proportional plasticity was the difference in trait values between treatments divided by the mean value in the predator-naïve treatment.

Two kinds of analyses tested for costs of plasticity, one in the plasticity experiment and the other in the competition experiment. The first followed van Tienderen's (1991) suggestion of checking for selection on both the phenotype itself and its plasticity. We accounted for nonlinear phenotypic selection by regressing tadpole mass against seven trait values (all except size) and the trait values squared. This analysis was repeated for both environments in the plasticity experiment. The resulting residual fitnesses were normalized and regressed against proportional plasticity. We ensured that a negative slope in this regression reflects a cost of plasticity. Body

size was not included in this analysis of costs in the plasticity experiment because body mass was our measure of fitness. Positive fitness residuals would indicate that for their body size these tadpoles were relatively heavy (relatively high body mass), meaning that these tadpoles had a dense body. We could not judge if a dense body would be connected to high or low fitness.

For the second kind of analysis, measures of both fitness and plasticity were differences between the two competing sibships in the competition experiment. We assessed relative competitive ability of every sibship, our measure of its fitness, as the difference between its rank-order appearance and the rank-order of sibships against which it was paired. Our measure of rank-order appearance was limited to the first four metamorphosing individuals because later metamorphs were not genotyped in the high-density competition experiment. We regressed the normalized fitness difference against the difference in proportional plasticity. A cost of plasticity would be revealed by a negative relationship, with the more plastic genotype suffering relatively low fitness.

One problem with our measure of fitness in the competition experiment is that age at emergence may trade-off against body mass. If individuals that metamorphose late are also large, then the order of emergence may be a poor indicator of fitness. This was not an issue for the low-density treatment, because separate analysis on differences in date and mass at emergence of all eight individuals gave similar results. In the high-density treatment, we checked the relationship between age and mass at metamorphosis for all individuals and found that late metamorphs were relatively small (estimate -17.0 mg/day; $F_{1,851}$= 53.4; $p<0.0001$). This suggests that our measure of fitness (rank-order appearance) detected tadpoles that were both early and large.

Results

Phenotypic plasticity

Rana temporaria in the plasticity experiment reacted to predators as expected based on previous studies (Van Buskirk 2002a). When exposed to dragonflies, tadpoles reduced activity by 48%, increased time spent hiding by 69%, increased tail fin depth by 11.3%, reduced tail length by 9.3%, reduced head depth by 2.2% and head length by 7.9%, and reduced body size by 14% (Table 1). Variation among sibships was highly significant for all traits except activity (Table 1). There was an overall (predator) treatment-by-sibship interaction in MANOVA (Wilks $F_{312,590}$=1.55; $p<0.0001$), reflecting differences among sibships in plasticity. In univariate analyses there was significant genetic variation in plasticity for head length and time hiding

(Table 1).

Correlation of plasticity between traits

Phenotypic plasticity was not positively correlated across all traits (Fig. 1), indicating that no aggregate measure of plasticity exists. The morphological traits had strongly correlated plasticities, with tail length negatively correlated with the other four traits. Plasticities in activity and hiding responded to predators in opposite directions and were therefore strongly negatively correlated. There was little association between plasticities in morphological and behavioral traits.

Costs of plasticity

In the plasticity experiment, there was little evidence for a cost of plasticity associated with any trait except tail length (Table 2; Fig. 2a,b). Sibships that strongly altered their tail length in response to *Aeshna* had somewhat low body mass in the predator treatment, after partitioning out variation in mass due to selection.

In the competition experiment, there was convincing evidence for costs of plasticity in body size and tail depth at both densities (Table 2). Sibships that showed strong increases in tail depth (Fig. 2c,d) and reductions in growth in the predator treatment metamorphosed later than their partners. Plasticity in head depth was more strongly costly at high density than at low density, but head length showed the opposite pattern. Coefficients for all traits except hiding were negative, and the positive coefficients for that single trait were small, indicating that costs of plasticity were higher than potential costs of homeostasis.

Discussion

Mixed evidence for costs of plasticity

The cost of phenotypic plasticity is paid by genotypes that have the capacity to be plastic, regardless of whether they express plasticity during their lives. An intuitively appealing theory outlines the role of plasticity costs in phenotypic evolution, yet few studies have successfully measured these costs. Our study highlights two possible explanations for the ambiguous empirical results.

The first is that costs are in fact small. In the plasticity experiment, where fitness costs were measured in units of body mass, the trait with the highest plasticity cost was tail length (Table 2). A sibship with maximal plasticity in tail length was estimated to suffer a 25%

reduction in body mass (1.3 SD units) relative to a sibship with the lowest plasticity. It could be argued that such a cost is not small, but one has to consider that these are the most extreme costs and for the trait with the by far highest costs. All other traits were associated with non-significant costs (≤ 5% of body mass). A similar comparison for the competition experiment suggested that sibships having high levels of plasticity emerged only three to four days later than sibships with low plasticity, even in the most costly traits such as tail fin depth and body size. Such a delay equals a 6.2-8.2% increase in time to metamorphosis. These plasticity costs are small enough that most previous experiments probably would not have detected them. In our study, the competition experiment revealed fairly clear costs because its measure of fitness was more sensitive than that used in the plasticity experiment. Relative fitness measures should allow detection of very small differences because they can extract fine distinctions between genotypes by reducing environmental background variation. Costs of plasticity may have occurred also in the plasticity experiment, but we were unable to detect them because absolute body mass is a less sensitive fitness measure.

A second reason for mixed results of earlier studies is that costs of plasticity are measured only for individual traits. This approach can produce confusing results if plasticities in different traits are uncorrelated. In fact, a typical result of earlier studies is that plasticity in one trait is costly while plasticity in another is beneficial (see Introduction). It seems unlikely that this reflects true differences among body parts in the cost of the capacity to be plastic. More likely, some traits are better than others at reflecting whole-organism plasticity. At a general level, we suspect that plasticity in morphology, especially tail fin depth, is most closely aligned with overall plasticity, because morphological traits consistently showed plasticity costs (Table 2). The trait that is likely the best in reflecting whole-organism plasticity is body size. Talking about plasticity in shape means mostly, talking about plasticity in size, which is why we corrected head and tail measures for size in the first place. If we do not size correct, almost all morphological traits reveal similar results that as for body size, showing good evidence for costs of plasticity, because they are closely correlated to plasticity in body size.

Correlated plasticities showed similar patterns in costs of plasticity. The highly positively correlated morphological traits, tail depth, head width, head length and head depth showed similar patterns in cost of plasticity in the competition experiment, though not all showed significant costs. In contrast, the only trait that was negatively correlated to the four positively correlated morphological traits, tail length showed ambiguous tendencies. The two negatively

correlated behavioral traits, activity and hiding showed contrasting patterns in costs of plasticity between the two experiments. When activity tended to indicate costs of plasticity, hiding indicated costs of homeostasis, and vice versa depending on the experiment.

Costs of plasticity and environmental stress

Several earlier studies report that costs of plasticity are greater in stressful environments (DeWitt 1998, Scheiner and Berrigan 1998, Tucic et al. 1998, Donohue et al. 2000, Dorn et al. 2000, van Kleunen et al. 2000, Steinger et al. 2003, Stinchcombe et al. 2004). Of the 13 studies cited in the introduction, ten observed costs of plasticity for at least some traits, and eight of these reported costs primarily within the environment that was judged to be more stressful (i.e., exposure to predators or competitors, plants under shade, saline conditions, or temperatures above the optimum). Two studies found that costs were greater in the less stressful environment. Overall, significant costs of plasticity were reported for 15 traits in the more stressful environment and only five traits in the less stressful environment.

There are two explanations for greater plasticity costs under stressful conditions. The first arises from a simple detection bias (van Kleunen et al. 2000). Even if absolute costs are identical under all conditions, they may be easier to detect in stressful environments where all individuals have lower fitness. This explanation assumes that, while mean fitness may be higher under benign conditions, a lower proportion of fitness variation is environmentally induced under stress. The second possibility is that plasticity costs may in fact be greater in stressful environments. The relationship between costs and environmental conditions depends on the mechanisms that reduce the fitness of more plastic genotypes. Stressful environments could exacerbate costs arising from resource limitation or allocation tradeoffs. Examples include the cost of maintaining sensory and regulatory structures necessary for plasticity, and information acquisition costs that necessitate extra energy or reduced foraging effort for sampling the environment (DeWitt et al. 1998). These kinds of costs may be severe when resources are limited, but compensating for them may be possible under favorable conditions.

The two explanations can be evaluated in our study by comparing results of treatments differing in stress. If plasticity costs increase under high stress, then we expect a steeper negative slope in the more stressful treatment (after removing effects of selection). If the detection probability improves with stress, then the variance around the regression coefficient should be smaller in the more stressful environment. These patterns were not upheld in our experiments (Table 2). Neither the regression coefficients nor the error estimates for those coefficients was

more negative respectively greater in the stressful (caged-predator and high-density) treatments. This suggests that the costs of plasticity and their probability of detection did not increase under stressful conditions, which contradicts the conclusions from some previous studies.

Costs of plasticity in various environments

It is remarkable that we find plasticity costs induced by just one form of environmental variation (predation risk). The cost of being plastic is caused by the capacity to express plasticity of many types, involving potentially many kinds of traits across all possible environments. An estimate based on just one kind of environment reflects costs associated with unmeasured reaction norms more likely if plastic responses to multiple environments are correlated. But this is not obviously true, and empirical studies are mixed. Reques and Tejedo (1997) found that toad sibships differed in their life history responses to density and pond drying, whereas Newman (1994) and Kaufman and Smouse (2001) reported that several traits showed correlated plastic responses to variation in two types of environments.

Conclusions

The costs of plasticity are probably weak in most organisms. The fact that plasticity is nearly ubiquitous in nature implies that costs are rarely strong enough to inhibit its evolution or maintenance, and most previous attempts to measure costs have been unsuccessful. Effect sizes observed in our study were relatively small, but costs were significant primarily because we focused on sensitive fitness measures. We suggest that a similar approach would be effective for assessing costs of plasticity in other organisms and induced by other kinds of habitat heterogeneity.

Acknowledgements

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Table 1: Summary of analyses of variance testing for effects of predator treatment, sibship, and their interaction on body size, morphological shape, and behavior. The predator treatment effect was tested over the treatment-by-sibship interaction. Entries in the table are F-ratios. Underlined values were nearly significant ($P<0.1$) and boldfaced values were significant (*, $P<0.05$; **, $P<0.001$; ***, $P<0.0001$).

		Trait							
Source of variation	df	Body size	Head length	Head width	Head depth	Tail length	Tail depth	Active	Hiding
Predator treatment	1	**43.2*** **	**24.3*** **	<u>3.4</u>	**48.1*** **	**17.2** **	**477.2*** **	**155.1*** **	**205.6*** **
Sibship	39	**2.2***	**6.6*** **	**3.4*** **	**5.3*** **	**3.6*** **	**4.3*** **	1.1	**4.1*** **
Treatment*Sibship	39	<u>1.5</u>	**1.8***	0.9	1.2	1.2	<u>1.41</u>	0.8	**2.7*** **

Table 2: Coefficients from linear regressions of fitness measures against plasticity in eight traits. Variance (SE) around the regression coefficients are given in brackets. All fitness measures are normalized and in SD units. Negative coefficients indicate a cost of plasticity. Mass was used as a measure of fitness in the plasticity experiment; rank-order age at metamorphosis was the fitness measure in the competition experiment. The sample size for all analyses was 40 sibships or competing pairs of sibships. Underlined values were nearly significant ($P<0.1$), and boldfaced values were significant (*, $P<0.05$; **, $P<0.01$).

Measure of fitness	Morphological traits						Behavioral traits	
	Body size	Head length	Head width	Head depth	Tail length	Tail depth	Active	Hiding
Mass								
No-predator treatment	--	-2.23 (8.65)	-0.51 (5.85)	0.12 (4.71)	-4.08 (13.71)	2.00 (3.44)	0.54 (0.71)	-0.07 (0.47)
Predator treatment	--	3.79 (8.64)	-5.19 (5.79)	-4.74 (4.66)	-23.90 (13.17)	-2.23 (3.44)	0.18 (0.71)	-0.17 (0.47)
Difference in age at metamorphosis (rank-order)								
Low density competition	**-4.88 (1.81)***	-20.56 (11.39)	-7.03 (8.08)	-10.68 (5.93)	2.73 (17.83)	**-9.70 (4.51)***	-0.71 (1.04)	0.76 (0.66)
High density competition	**-4.23 (1.75)***	-6.23 (11.27)	-6.91 (7.71)	**-11.99 (5.56)***	-11.03 (16.91)	**-11.43 (4.16)****	-0.71 (1.00)	1.04 (0.61)

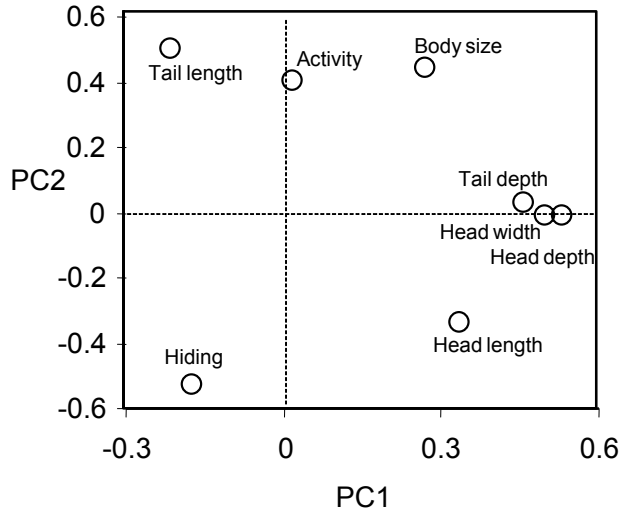

Fig. 1: Principal component analysis on plasticity in body size, morphological shape, and two behavioral traits. PC1 explains 32.6% and PC2 explains 25.3% of the variance. The position of traits in the figure reflects their coefficients on the two axes.

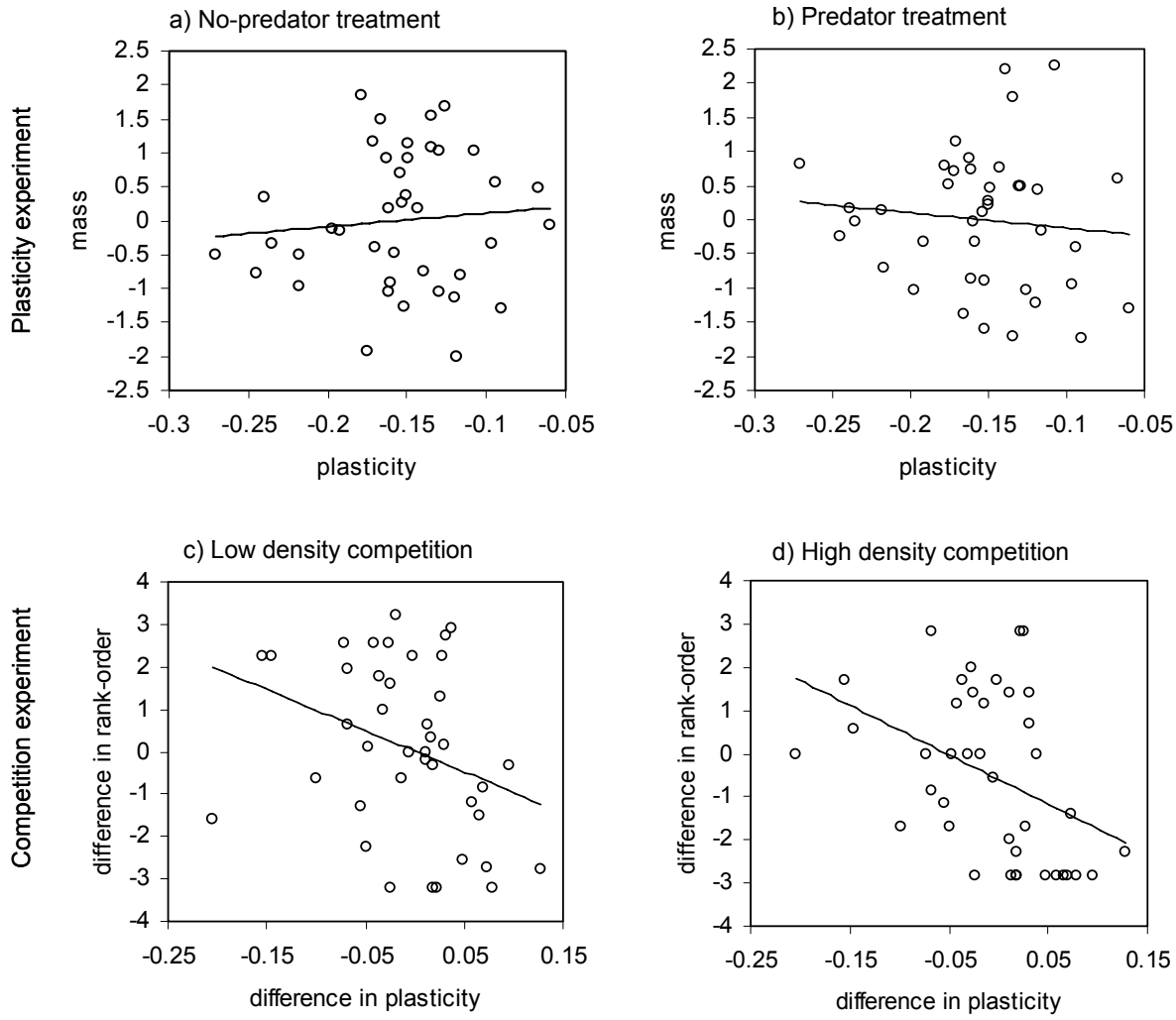

Fig. 2: Costs of predator-induced plasticity estimated by regressing fitness measures against plasticity in tail depth, for 40 sibships of *Rana temporaria* tadpoles. Fitness in the plasticity experiment (a and b) was assessed by tadpole mass after partitioning out variation due to selection. For the competition experiment (c and d) the difference in rank-order time until metamorphosis between two competing sibships is plotted against their difference in plasticity. Each point represents a sibship (a and b) or a pair of competing sibships (c and d). Negative correlations indicate costs of plasticity. Table 1 gives results of significance tests.

CHAPTER 2

OPTIMIZING TIME AND RESOURCE ALLOCATION TRADE-OFFS FOR INVESTMENT IN MORPHOLOGICAL AND BEHAVIORAL DEFENSE

Ulrich K. Steiner and Thomas Pfeiffer

Abstract

Organisms are often confronted with time and resource allocation trade-offs. Time allocation trade-offs partition time, e.g. between foraging effort to acquire resources and behavioral defense. Resource allocation trade-offs partition the acquired resources between multiple traits, such as growth or morphological defense. We develop an integrated optimality model with prey organisms in mind, where we comprise time and resource allocation trade-offs for multiple defense traits. In the model, we maximize survival during ontogeny by optimizing investment in behavioral and morphological defense for different environments. We compare the results of the integrated model with simplified versions that optimize only a single defense trait. We show that behavioral and morphological defense compensate for and augment each other dependent on predator densities and effectiveness of the defense mechanisms. The response patterns to predator density and resource availability are more pronounced in the simplified settings compared to the integrated optimization. This implies that the evolution of integrated traits is favored and simplification overestimates the effects on population and community dynamics. When incorporating time constraints, the model shows a peak investment in optimal morphological and behavioral defense at intermediate resource availability. This does not occur in the absence of time constraints.

Introduction

Organisms have evolved a variety of phenotypes and life-histories to solve and mediate conflicting demands. Trade-offs play a major role for such evolutionary processes. Phenotypic integration, the ecology and evolution of complex phenotypes and life-histories receive increasing interest (Rundle and Brönmark 2001, Pigliucci 2003, Cotton et al. 2004, Mikolajewski and Johansson 2004). Two of the most fundamental trade-offs are resource and time allocation trade-offs. Time allocation trade-offs allocate limited time to various time-consuming traits or activities, such as mating or courtship activity, foraging effort, or behavioral defense (Lima and Dill 1990, Lima 1998, Angilletta et al. 2003). Resource allocation trade-offs, where resources rather than time is limited, distribute limited resources between various competing traits, such as growth, maintenance, reproduction, storage or morphological and physiological defenses (Coley et al. 1985, Herms and Mattson 1992, Perrin and Sibly 1993, Angilletta et al. 2003).

Prey organisms are typical examples facing conflicting demands by being confronted with time and resource allocation trade-offs. Responses of prey to predators are used in the following study as example to explore the two trade-offs. Prey organisms mediate predation by multiple integrated morphological and behavioral defense mechanisms. In many prey organisms, time for foraging is trading off against time spent for behavioral defense, such as seeking shelter. Foraging activity requires movement, which increases the encounter rate with predators and therefore increases the predation rate (Werner and Anholt 1993). Acquired resources have to be allocated to competing functions such as growth and morphological defenses, e.g. higher crests in *Daphnia* (Barry 1995).

Life-history theory predicts that the allocation of time and resources to traits is determined by the optimization of the net fitness for the individual (Stearns 1992). The expression of each trait is expected to depend on its cost-benefit ratio in the current environment, hence optimal time and resource allocation strategies are expected to vary between environments. Harsh environmental conditions, such as environments with high predator densities or food shortage, are expected to intensify the trade-off between growing and defending at the same time. Low resources are expected to select for high foraging effort, because more time is needed for searching for and harvesting the scarce resources. High predator density is expected to select for better defended individuals showing low foraging effort and high investment in behavioral and morphological defense. The investment in behavioral and morphological defense is predicted to be weaker when resources are scarce than when they are abundant, because allocation in life-

saving mechanisms such as maintenance are more or less fixed in their absolute amounts that have to be allocated to them. Hence, such life-saving mechanisms should become proportionally less resource demanding when resources get more abundant (Clark and Harvell 1992).

Time allocation trade-offs have received substantial empirical interest in predator-prey systems, with dragonflies, fish and crayfish as predators and tadpoles, damselflies and snails as prey (McCollum and Van Buskirk 1996, Van Buskirk and McCollum 2000a, Rundle and Brönmark 2001, Stoks 2001, Altwegg 2002, Benard 2004, Brodin and Johansson 2004, Cotton et al. 2004, McPeek 2004, Mikolajewski and Johansson 2004). Various names have been used for such activity based trade-offs, such as acquisition trade-off (Angilletta et al. 2003) or growth-predation risk trade-off (Brodin and Johansson 2004, McPeek 2004). Other empirical studies have explored resource allocation trade-offs for various predator-prey systems (Clark and Harvell 1992, Barry 1995, Van Buskirk 2000, Lankford et al. 2001, Cotton et al. 2004, Mikolajewski and Johansson 2004). In plant-herbivore systems resource allocation trade-offs were often explored for secondary metabolites as defense mechanisms (Ohnmeiss and Baldwin 2000, Cipollini et al. 2003, Biere et al. 2004). Various environmental effects, such as time constraints (Blanckenhorn 1998, Altwegg 2002) and food/nutrient availability (Blanckenhorn 1998, Anholt et al. 2000, Heil et al. 2002) have been explored in relation to predation.

A number of theoretical models have been developed for time allocation trade-offs in predator-prey systems (Abrams 1984, Houston et al. 1993, Werner and Anholt 1993, McNamara and Houston 1994, Yearsley et al. 2002, Abrams 2003). Various studies model resource allocation trade-offs in predator-prey systems for food web dynamics, size specific predation, growth or reproduction predation trade-offs and cost-benefit ratios of morphological defenses (Clark and Harvell 1992, Taylor and Gabriel 1992, Day et al. 2002, Irie and Iwasa 2005). Similar resource allocation trade-off models for plant-herbivore systems exist for physiological trade-offs of secondary metabolites (Coley et al. 1985, Tuomi et al. 1991, Herms and Mattson 1992, Iwasa 2000).

Both, time and resource allocation trade-offs are not independent from one another. Foraging activity determines how much resources can be acquired. Hence it determines the amount of resources that can be allocated to the various traits competing for resources. Conversely, the expression of some traits might have an indirect effect on optimal foraging activity. Individuals respond to reduced predation probability by increasing their (foraging) activity (McCollum and Van Buskirk 1996, Brodin and Johansson 2004). Traits that reduce the

predation probability, such as morphological defense and weight or size (Arendt 1997) may allow an individual to increase the optimal foraging activity. Hence, the two kinds of trade-offs affect each other.

In this article we develop a model that combines time and resource allocation trade-offs within an optimization framework. Previous models have only incorporated one of the trade-offs. In a first step, we set the model parameters to calculate the optimal foraging activity (hereafter activity) in the absence of predation where no morphological and behavioral defense is necessary. In a second step, we determine the optimal morphological defense under predation for a constant activity calculated in the first step. In a third step, we assume that only activity is optimized under predation, but no morphological defense occurs. Finally, in a fourth step, we simultaneously optimize both activity and defense in the presence of predation. The model is explored along two environmental gradients, predator density and resource availability, and for the effectiveness of the morphological defense.

The four steps allow us to compare single-trait optimization of only morphological defense (step 2) or behavioral defense (step 3) with the simultaneous optimization of both, morphological and behavioral defense (step 4). The model also allows us to assess the investment in morphological and behavioral defense by comparing the optimal strategy in the absence of predation (step 1) with the optimal strategy in the presence of predation (step 4). The difference in trait expression between the no-predator (step 1) and the predator environment (step 4) is defined as the predator-induced plasticity in the modeled (defense) traits and reflects directly the investment in morphological and behavioral defense (Tollrian and Harvell 1999).

Model background

We design the model with developing and growing (immature) individuals in mind. We choose such individuals for two reasons: first, developing and growing individuals are normally prone to higher predation rates compared to adults and show intensified conflicting demands between growth and defense (Arendt 1997). Second, choosing immature individuals allows to simplify the model, because we can ignore any allocation towards reproduction.

Our optimality model is based on life-history theory, where survival can be seen as a surrogate of fitness, because reproduction does not play any role for immature individuals. Life-history theory predicts that selection should favor a strategy that maximizes survival for the period that is required to grow or develop from the initial weight or developmental state to the final weight or state (Stearns 1992). The initial state or weight could be the state or weight at

hatching or birth. The final developmental state or final weight that has to be reached might be adulthood or adult weight or some state or weight at which individuals overwinter, metamorphose, pupate or enter the next instar.

The time needed to reach this final state was often related to high fitness and survival. This applies for organisms that have to reach the next instar before the end of the season, such as many insect larvae or metamorphosing vertebrates, and to many other organisms were being large and early is correlated with high survival probabilities to first reproduction and high fitness (Arendt 1997). We therefore first present a model that does not include time constraints and then extend the model for time constraints which account for fitness payoffs related to increased time to reach the final state.

The model

In our model, the key parameter (objective) for which we optimize is survival to final weight, with final weight equivalent to final state. Survival depends on two factors, first the background mortality, which is mortality independent from predation, and second, the mortality due to predation. Latter is mediated by the foraging activity, the size and the morphological defense of the individual. In the course of growing, not only the weight but also the predation probability and the morphological defense traits changes. In the model, the expression of the morphological defense trait depends on the amount allocated towards morphological defense; morphological defense is thought to continuously increase during the growth phase. Mortality and hence survival is also affected by environmental factors such as predation density and resource availability. These effects of environmental conditions are discussed after introducing the model in more detail.

For the model we use three differential equations to describe weight g, allocation into morphological defense n, and the probability of survival p:

$$dg/dt = (1-\delta)\, R_{UP} \qquad (1)$$

$$dn/dt = \delta\, R_{UP} \qquad (2)$$

$$dp/dt = -m\, p \qquad (3)$$

Equation 1 describes the growth of an organism (excluding defense traits). Equation 2 describes the dynamics of morphological defense traits. The parameter δ describes how much of the acquired resources R_{UP} are invested into morphological defense. Equation 3 describes the probability of survival p of an organism experiencing mortality m. We assume that at the initial

state (*t=0)* the weight is given by the initial weight $g(t=0)=g_0$, the initial morphological defense is $n(t=0)=0$, and the probability of being alive is $p(t=0)=1$.

Resource uptake

We assume that resource uptake R_{UP} depends on the weight g of an organism, its activity α, and the resource availability R in the environment:

$$R_{UP}= gR\alpha(1-\alpha)/(1+R\alpha) \qquad (4)$$

The term $(1-\alpha)/(1+R\alpha)$ introduces a cost for high activity and a reduction in the efficiency of acquiring resources at higher resource availability. This assumption implies that the efficiency of harvesting resources decreases with increasing activity and resources. Resting time is required for processing and digesting food and organisms have an upper limit in harvesting food even if there is more food available, this is simply due to physiological and morphological constraints. Without such a limit, one could not provide *ad libitum* food to organisms. For simplification, we do not include terms for maintenance and storage, which would also compete for resources. We assume that allocation in maintenance and storage is subsumed under equation 1, allocation towards growth.

Mortality

Mortality m is given by the following equation:

$$m= m_B+\alpha P/(g+(1+E)n) \qquad (5)$$

Here, m_B describes a constant predation-independent background mortality. The remaining term describes mortality due to predation (predation probability). Predation probability depends on the activity α, because high activity increase the encounter rate with predators and movement attracts predators, both resulting in increased predation rates (Werner and Anholt 1993). It also depends on the predator density P, which enfolds the number of predators in the environment and how dangerous those predators are. Furthermore, it is affected by the state dependent weight g, because larger individuals are mostly depredated on less often (Arendt 1997). Finally, predation probability depends on the morphological defense n, and how much more efficient this morphological defense E is in relation to the reduction of predation probability due to weight g. Equations (1-5) allow to calculate the probability of survival from the initial state to the time when the final weight is reached. For non-time constrained conditions, fitness w is assumed to be proportional to survival and is maximized in our model.

Optimizing activity in the absence of predation (step 1)

In the first step we determine the optimal activity in the absence of predation. In an environment without predation there is only a constant background mortality. Therefore, maximizing survival is equivalent to minimizing the developmental time to the final weight, which is equivalent to maximizing the growth rate $(1-\delta) R_{UP}$. As can be expected, in the absence of predation investment to morphological defense at the cost of a reduced growth rate is not beneficial. Maximizing the resource uptake R_{UP} results in an optimal activity of $\alpha=1/(1+\sqrt{1+R})$. This implies that in the absence of predation, the optimal activity depends only on the availability of resources.

Single-trait optimizations

Optimizing morphological defense in the presence of predation (step 2)

In the second step we determine the optimal morphological defense in an environment with predation, assuming there is no plasticity in activity. The activity is therefore the same as determined in an environment without predation (see step 1). We use equation 1-5 to numerically calculate the survival at the final weight for any investment into morphological defense. This allows calculating the optimal investment into morphological defense.

Optimizing activity in the presence of predation (step 3)

In the third step we determine the optimal activity in an environment with predation, given there is no morphological defense. In this step predation probability is only mediated by activity and weight. Without morphological defense, the mortality formula (equation 5) can be simplified to $m= m_B+\alpha P/g$. Again, we use equation 1-5 to numerically calculate the survival at the final weight and determine the optimal activity.

Integrated-trait optimization

Optimizing activity and optimal morphological defense in the presence of predation (step 4)

In step 4 we assume that in contrast to step 2 and 3, both activity and morphological defense are plastic. We thus optimize both activity and morphological defense simultaneously using equations 1-5.

The above four steps are explored for their response in survival, activity, and investment in morphological and behavioral defense along a predation (step 2-4) and a resource availability gradient (step 1-4). We describe correlations of investment in behavioral and morphological

defense in the context of varying predator densities and varying effectiveness of morphological defense. We compare the single-trait optimization settings (step 2&3) with the integrated optimization for both defense traits (step 4). This comparison allows to assess how phenotypic integration affects the optimal strategy for investment in behavioral and morphological defense in comparison to strategies based on only one defense trait. Finally, we extend our model by including time constraints and test for robustness of the model. Note, investment in behavioral defense is equivalent to predator-induced plasticity in activity and is calculated as the difference between the activity in the absence of predation (step 1) to activity in the presence of predation (step 3 or 4). The optimization procedure is always done with the parameters listed in table 1.

Results of the unconstrained model

The comparison between the single-trait optimizations (step 2&3) and the integrated-trait optimization (step 4) reveals a general pattern; the single-trait optimizations always show lower survival (Fig.1), lower activity and higher investment in morphological (Fig. 4) or behavioral defense than the integrated optimization. Survival differences between the integrated and the single-trait optimizations are stronger at intermediate environmental conditions than at harsh (i.e. low resource, low predation) or benign (i.e. high resource, high predation) conditions (Fig. 1).

High predator densities decrease survival (Fig. 1b) and select for lower activity and more investment in behavioral and morphological defense (Fig. 2). Increased availability of resources increases survival (Fig. 1c) and selects for lower activity and reduced investment in morphological and behavioral defense (Fig. 2). The decrease in activity with increasing resources is stronger in the no-predator environment (step 1) compared to the predator environment (step 3&4). Thus, the investment in behavioral defense decreases with increasing resource availability (Fig. 2).

Increased effectiveness of morphological defense increases survival in the integrated optimization (step 4) and morphological defense optimization (step 3, Fig. 1a). Interestingly, increasing morphological defense effectiveness selects for investment into morphological defense but against investment in behavioral defense in the integrated optimization (step 4, Fig. 3). The latter is an indirect effect of the increased investment in morphological defense at higher defense effectiveness, because the decrease in investment in behavioral defense only occurs in the integrated optimization (step 4) but not in the activity optimization (step 3). At very high morphological defense effectiveness investment in defense decreases in the integrated

optimization (step 4) and the morphological defense optimization (step 2) (Fig. 4), while investment in behavioral defense continues to decline.

If individuals or environments differ in the effectiveness of morphological defense, the investment in behavioral and morphological defense are negatively correlated, so that investment in behavioral defense increases to compensate when the morphological defense is less effective (Fig. 3; different darkness of different symbols, system shifts along the thin black lines). In contrast, if environments or individuals differ in perceived predation, a positive correlation between investment in behavioral and morphological defense occurs, with greater investment in both kinds of defense in more risky situations (Fig. 3; system moves between symbols of the same color, but different size). Thus, morphological and behavioral defenses do not necessarily augment each other, but can sometimes compensate for each other.

Time constraints

In the above set of models we have not included any time constraints. Only an indirect predation and background mortality effect selects for fast growth. Individuals that grow slowly and hence need more time to reach the final weight are more likely to die due to background mortality and - in a predator environment - they are longer exposed to predation and hence have reduced survival. Many organisms pay extra costs of slow development or growth or are time constrained by e.g. the growing season. Larger individuals often show increased survival probabilities over the winter or during other harsh conditions and also have higher prospects of future reproductive output (Arendt 1997). Slowly developing and growing individuals might also pay extra fitness costs by delaying reproduction. Thus, we extended our original model and incorporated extra fitness costs of slow growth in the following model. In this time-constrained model, optimization was done for a combination of survival p and the time point t_{final} of reaching the final weight. Overall fitness w is given as:

$$w = (S^4/(S^4 + t_{final}^4))p(t=t_{final}) \qquad (6)$$

The growing season or time constraints for the organism is determined by the end of the optimal growing season S, which effects the fitness at the end of the season or the time when an individual reached the final weight, hence it incorporates a cost for slow growth. At the beginning of the growing season $(t=0)$, there is little reduction in fitness with each time step t, once an individual approaches the end of the season S, there are high fitness costs, and each time step t that is required to reach the final weight causes high extra fitness costs. The time fitness function

describes a negative sigmoid relationship, starting with high fitness at the beginning of the season to low fitness after the end of the season.

Results of the time-constrained model

The time-constrained model reduces fitness for all combinations of parameter settings. A general pattern of this model and therefore of time constraints is that it selects stronger for growth than the unconstrained model. The effects of defense effectiveness and predator densities are similar to those in the model without time constraints, except that there is less investment in behavioral and morphological defense (darker grey at high investment in Fig. 2 c&d compared to Fig. 2 a&b) and a slightly higher activity. The activity in the no-predator environment (step 1) does not vary between the time-constrained and the unconstrained model.

The most striking difference between the time-constrained model and the unconstrained model is the response to the resource availability gradient (Fig. 2). In the unconstrained model, investment in morphological and behavioral defense decreases with increasing resources (Fig. 2 a&b), but in the time-constrained model, defense first increases and then decreases with increasing resources (Fig. 2 c&d). Under time constraints when resources are scares, individuals have to invest everything in growth to reach the final weight before the season ends and cannot afford to invest in defense without paying very high fitness costs due to delayed reaching the final weight. Such a peak in defense at intermediate resource availability is less pronounced at very low and very high predation densities (Fig. 2 c&d). Investment in behavioral and morphological defense do not peak at the same resource availability. Thus, both defense types were not directly correlated. At high resource availabilities, survival, activity and defense do not differ between the time-constrained models and the unconstrained models, because high resource availability allowed a fast growth and prevented any effect of time constraints (no difference in grey shadings at high resource availability, Fig. 2).

The patterns of responses are robust to variation in time constraints *S*. Smaller *S* values (shorter growing seasons) select for growth and hence for higher activity and lower investment in behavioral and morphological defense. The models are also quite robust to other changes, such as the costs of high activity and changes in the efficiency for the resource uptake with resource availability. Reduced costs of activity and reduced decrease in the efficiency for the resource uptake at high resource availability, select for higher activity, for more investment in behavioral and morphological defense and for higher survival in comparison to the original time-constrained model.

Discussion

We theoretically explore optimal solutions to simultaneously operating trade-offs. Time and resource allocation trade-offs are widespread in animals. Integrating both mechanisms, behavioral and morphological defense reveals a theoretical understanding of compensation and co-specialization of multiple traits. Both mechanisms, behavioral and morphological defense complement one another in different ways depending on environmental conditions, by either compensating or augmenting each other. The compensation effect is shown by the negative correlation between behavioral and morphological defense when effectiveness of morphological defense varies, so that investment in behavioral defense increases to compensate when the morphological defense is less effective. The augmenting is shown by positive correlations with greater investment in both kind of defense in more risky situations. The single-trait optimization did not reveal a compensation effect seen for the integrated optimization.

Previous models have used dynamic modeling (Bellman 1957, Mangel and Clark 1988) to solve conflicting demands in time and resource allocation trade-offs (Clark and Harvell 1992, Houston et al. 1993, Werner and Anholt 1993, Iwasa 2000). Such models allow state dependent optimal activity and state dependent optimal investment in defense. Even though, our model is limited to some extent, because it does not allow such state dependent responses, it allows to integrate time and resource allocation trade-offs and optimize more than one defense trait simultaneously, which is very difficult in a dynamic programmed model.

Compensation between behavioral and morphological defense

Several empirical studies have shown compensation effects of behavioral and morphological defenses, where morphologically less defended species exhibit stronger behavioral defense (Rundle and Brönmark 2001, DeWitt and Langerhans 2003, Cotton et al. 2004, McPeek 2004, Mikolajewski and Johansson 2004). Unfortunately, these studies do not allow a direct comparison with our model. Our model explores trait integration along environmental gradients, however empirical studies along such gradients are lacking. In the empirical studies, defense mechanisms are very different. In one study thinner shelled snails which are more vulnerable to predation showed greater behavioral responses (Rundle and Brönmark 2001), while in another study shell strength had no effect but shell shape did (Cotton et al. 2004). Within studies results were not always clear cut. Three dragonfly species compensated or augmented each other in their behavioral and morphological defenses, depending on which pair of species was compared (Mikolajewski and Johansson 2004).

Integrating multiple traits

Without integrating several traits, patterns might not be adequately interpreted in theory and for empirical data. Not only behavior and morphological defense traits are integrated, also other traits such as growth interact with our focal defense traits. Every investment in defense, irrespective of its behavioral or morphological nature, trades off against growth and hence against time, here to season end. Empirical studies support that our theoretical understanding does not always fit with the complex integrated traits working in nature. A study on a number of damselfly species showed that faster growing species exhibited higher activity and were more vulnerable to predation compared to species showing less growth, less activity and being less vulnerable to predation (McPeek 2004). All these findings are in line with the predictions and assumptions of our model. However, contrary to expectations the two groups of species that differ in growth showed no difference in feeding or ingestion rates. The fast growth of the first group was achieved by a higher conversion rate of food under predation. Here, without integrating physiological traits, the interpretation of the data would have been difficult. This again shows how important it is to integrate several traits and not to study only atomized traits.

Integrating more traits simultaneously, in our model, produces less pronounced response patterns to predation and several environmental gradients than the single-trait optimization. Such a weakening of responses can explain why some considerable effects predicted by simple models might be hard to detect in empirical or experimental data, because other mediating effects, which might not be in focus of the experiment, weaken the expected pattern. However, the single-trait optimizations reveal, apart of not detecting some compensation effects, similar patterns as the integrated optimization. Single-trait optimization and integrated optimization showed similar responses in survival to various gradients (Fig. 1), peak investment in morphological defense at intermediate defense effectiveness (Fig. 4), increased behavioral and morphological defense in response to higher predator densities and reduced investment in morphological and behavioral defense in response to increased resource availability. All these results show that many of the previous models optimizing only one trait make useful predictions.

Evolution of integrated traits

Evolution favors multiple integrated (defense) traits. This is demonstrated by the higher survival in the integrated optimization, despite less pronounced defense responses compared to the single-trait optimization. It explains why we commonly see phenotypic integration and the evolution of multiple, integrated defensive traits in nature (Lima 1998). Selection for integrated

traits is stronger at intermediate environmental conditions than at harsh (e.g. low resources, high predator densities) or benign (e.g. *ad libitum* resources, low predator densities) conditions, because survival differences between the integrated and the single-trait optimizations are more pronounced at intermediate environmental conditions. This shows that experiments done at *ad libitum* food might reveal different results than experiments done under more natural conditions, because most organisms do not experience and evolve under *ad libitum* food in nature.

Trait integration effects on population and community dynamics

Single-trait optimizations overestimate effects of time and resource allocation trade-offs on population and community dynamics. Integrated optimization allows to invest less in defense without increasing predation probability, shown by a higher survival in the integrated optimization compared to the single-trait optimization. The cost-benefit ratio of investment in defense changes between integrated and single-trait optimization, which has great implications for prey population dynamics, but also predator dynamics are affected because vulnerability of prey changes. As a consequence, the selection for phenotypic integration and against single-trait mechanisms alters implications for community and population dynamics. Thus, single-trait models of time and resource allocation trade-offs (Abrams 1984, 1990, Werner and Anholt 1993, Abrams 2000) likely overestimate community and population effects.

Time constraints

Implementing time constraints results in changes of the optimal strategies for mediating predation. In the time-constrained model, investment in behavioral and morphological defense peaked at intermediate resources. When resources are very scarce investment in growth is prioritized over defense. Fitness payoffs for delayed reaching of the final weight are more severe than any fitness reduction due to lacking defense. At intermediate resource availability, high investment in defense pays off, because resources do not allow a growth rate to flee predation by reaching the final weight quickly, and individuals are exposed to predation for a long time. When resources are abundant individuals can reduce the activity and still gain enough resources for a high growth rate. Only little investment in morphological defense pays off, because the reduction in activity is the most efficient defense mechanism. Under high resource availability the strategies of defense do not differ between the time-constrained and unconstrained model. Peak patterns of investment in defense have been predicted by some models (Tuomi et al. 1991, Herms and Mattson 1992). Only a few experiments have shown such patterns for morphological (Barry

1995) and behavioral defense traits (U.K. Steiner unpublished) in animal systems. A reason why such patterns have rarely been reported, even though they might commonly exist, is that most experiments on defenses or reaction norms assessed effects only for two conditions and not along continuous gradients, hence these experiments were unable to detect nonlinear patterns (Angilletta et al. 2003).

The difference between the time-constrained and unconstrained model highlights another problem; laboratory experiments without cues about seasoning or time constraints are expected to produce different results than experiments under more natural conditions, under which the traits and trait responses have evolved. The unconstrained model shows a continuous decrease in investment in defense with increasing resource availability, while the time-constrained model shows a peak of investment at intermediate resource availability.

Response to environmental variation

Most of the changes of investment in morphological and behavioral defenses in response to environmental variation are as expected. Under conditions that drive the optimal life-history strategy towards increased growth (e.g. time constraints) predation has only a minor effect on survival and on the optimal strategy. Growth rate is the main factor that has to be maximized. Hence, only little is or can be invested in morphological and behavioral defense and much is allocated towards growth.

An unexpected result emerges for the investment in morphological and behavioral defense with increasing resources. We expected that lower resource availability would select for growth and against investment in morphological and behavioral defense; but investment in behavioral and morphological defense decreases with increasing resources. Irrespective of predation there is a decline in activity with increasing resource availability. This decline is more pronounced in the no-predator environment, which leads - at first glance - to a confusing pattern in investment in behavioral defense. Individuals are behaviorally better defended at high resources compared to low resources despite reduced investment in behavioral defense (predator-induced plasticity in activity). Several studies showed that tadpoles reduced their activity when resource availability is high (Anholt and Werner 1995, Laurila et al. 1998, Anholt et al. 2000, Peacor 2002), although none of the studies revealed evidence that this reduction of activity was more pronounced in a no-predator environment.

The decline of morphological defense with increasing resources is also unexpected, though such patterns have been discussed in some plant systems (Myers and Bazely 1991). We

expected that at high resource availability more allocation in morphological defense should be made, because proportionally little resources would have to be allocated to growth and other life saving mechanisms such as maintenance and storage. Contrasting the expectations, investment in morphological defense decreases with increasing resources. A likely explanation is that at low resources individuals are longer exposed to predation and therefore have to defend themselves better (if there are no time constraints). Morphological defense seems to be the most beneficial defense option under such limited resource conditions. This might indicate that per unit of mortality reduction morphological defenses are cheaper than behavioral defenses.

Increased effectiveness of morphological defense causes increased investment in morphological defense and selects against behavioral defense. Increased morphological defense allows to express higher activity, because predation probability can be more efficiently reduced by increased morphological defense than by investing in behavioral defense. Hence, behavioral defense becomes proportionally less effective with increased effectiveness of morphological defense. At low morphological defense effectiveness, predation is mainly or only mediated by activity and not by morphological defense, while when morphological defense gets more effective, predation is mainly mediated by morphological defense and activity does not mediate predation strongly. A decline in investment in morphological defense occurs only at very high defense effectiveness (Fig. 4). Such a decline is expected, because only little has to be invested in defense to greatly reduce predation probability.

Implications

Our models have implications for studies on the evolution of trait integration to mediate the impact of predators on mortality and potentially other fitness threatening or conflicting factors. The results reveal that population and community dynamics alter with increasing number of integrated traits, and theoreticians and experimental biologists should be aware of such integrated and interacting traits when exploring environmental gradients, time constraints and reaction norms in an optimality context. We tried to make our model as simple as possible, hence we do not call for developing more complicated models, but we would like to alert theoreticians and empiricists to be aware that simplification might lead to alluring expectations of strong effects that will be much weaker when integrating more traits.

Our findings can be generalized with some cautions to several allocation trade-offs. Simplification by atomizing behavioral, physiological or morphological traits of various time and resource allocation trade-offs should have similar problems in underestimating effects mostly at

intermediate environmental conditions. We believe that studies that allow the detection of nonlinear responses for more than one environment and for more than a single trait would hold great potential to improve our understanding of the evolution of complex phenotypes and life-histories. Studies should be conducted under conditions that are close to natural conditions under which evolution took place. We are aware that conducting such experiments will be challenging, and time-consuming, but we believe that the results of such experiments will reward the effort.

Acknowledgements

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Table 1: Parameters used in the optimization procedure. The exploration of the model was limited to values where the greatest effects were observed.

Parameter	Parameter description	Value
g_0	Initial weight	1
$g(t=t_{final})$	Final weight	5
g	Weight while growing	equation 1
n	Morphological defense $n(t=0)$=0	equation 2
p	Probability of survival $p(t=0)$ =1	equation 3
t	Time	variable
m	Mortality	equation 5
m_B	Background mortality	0.01
R_{UP}	Resource uptake	equation 4
R	Resources availability	0.25-16 (Fig. 1&3); 1 (Fig. 2)
P	Predator density	0.125-8 (Fig. 1-3)
α	Activity	optimized
δ	Allocation into morphological defense	optimized
E	Effectiveness of the morphological defense	8 (Fig. 2); 2-128 (Fig. 2&4)
S	Season end (time-constrained models)	30
w	Fitness	target of optimization; equation 6

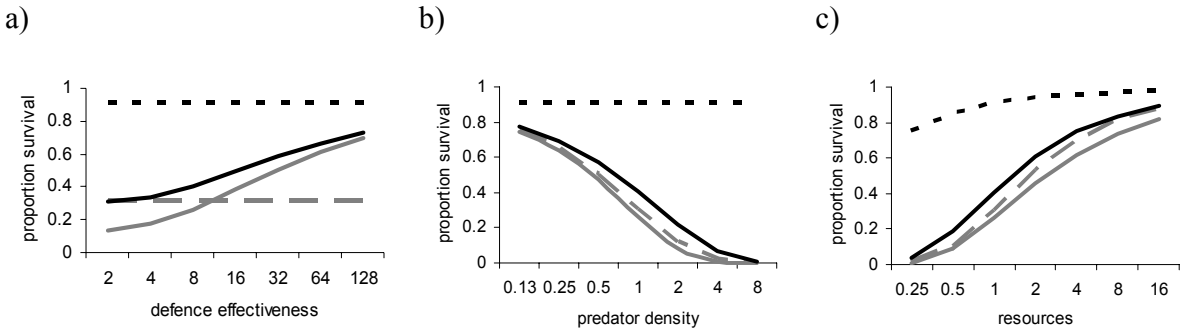

Fig. 1: Proportion of survivors at various defense effectiveness (a), predator densities (b) and resource availabilities (c). Optimized in a no-predator environment (step 1; dotted black lines), for only morphological defense (step 2; solid grey lines), for only behavioral defense (step 3; hatched grey lines), and for behavioral and morphological defense simultaneously, the integrated optimization (step 4; solid black lines). Mortality due to predation is the difference between the dotted black line and the solid black line for the integrated optimization and the dotted black lines and the grey lines for the single-trait optimizations.

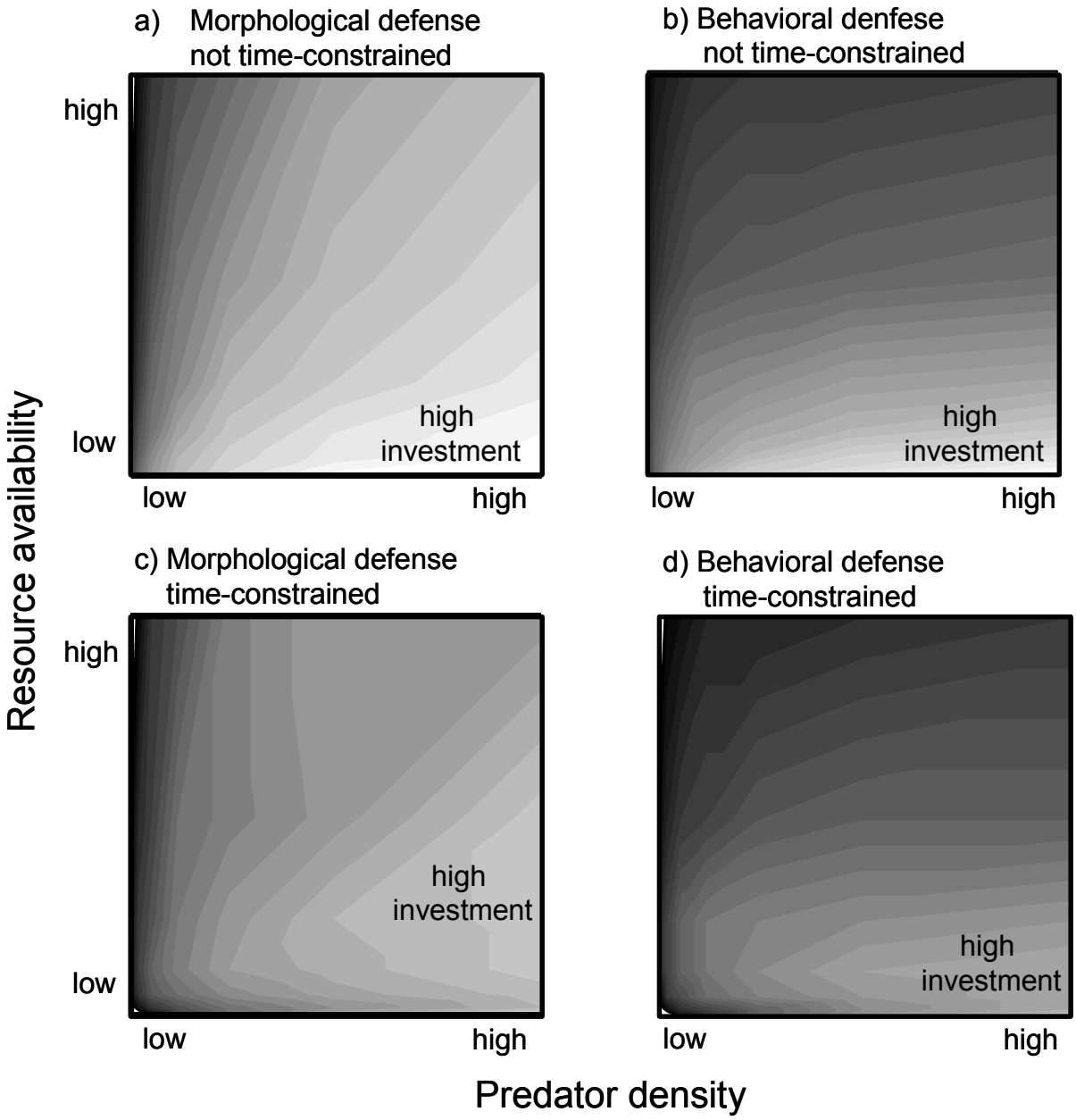

Fig. 2: Optimal investment into morphological (a & c) and behavioral (b & d) defense under different predator densities and resource availability, without (a&b) and with (c&d) time constraints. Light grey shadings reflect high investment in defense.

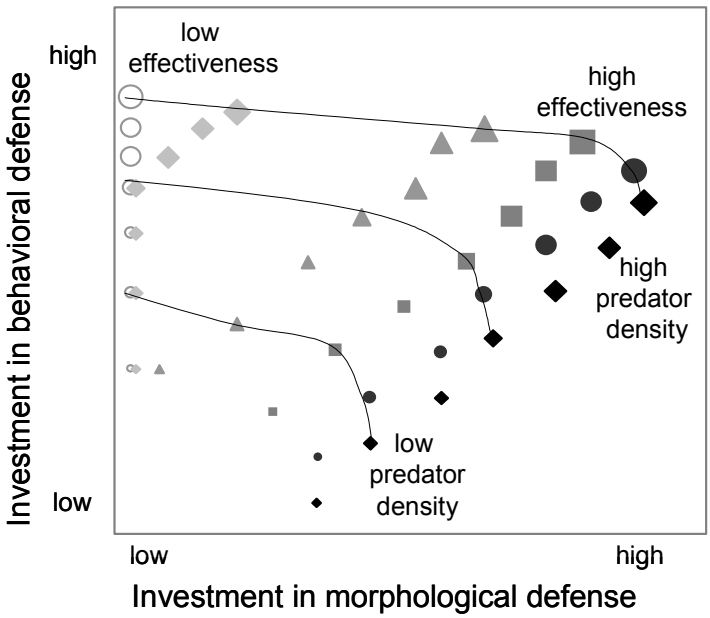

Fig. 3: Correlations among optimized investment in behavioral and morphological defense along a predator density and effectiveness of morphological defense gradient. Each symbol represents an optimal solution for the integrated optimization (step 4) under different predator densities (symbol sizes) and effectiveness of morphological defense (darkness and different type symbols along thin black lines).

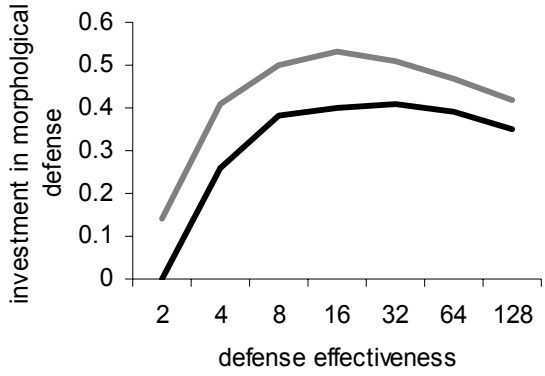

Fig. 4: Investment in morphological defense at different defense effectiveness optimized for only morphological defense (step 2; grey line), and for behavioral and morphological defense simultaneously, the integrated optimization (step 4; black line).

CHAPTER 3

INTERACTIVE EFFECTS OF PREDATOR-INDUCED DEFENSES AND TRAIT COSTS ALONG A RESOURCE GRADIENT

Ulrich K. Steiner

Abstract

Organisms have evolved multiple defensive traits to mediate predation risk. The expression of defensive traits is determined by the trade-off between the effectiveness of defense in each trait and fitness costs of building the trait. The costs in responding to predators caused by defensive traits have to be compensated by fitness traits and should be measurable by reduced expression in fitness traits. If defensive traits vary in their costliness, shifts in trait expressions should occur with varying resource availability. Models based on acquisition and allocation trade-offs predict different outcomes along a resource availability gradient. For behavioral defenses and costly defensive traits, an increased investment in defense is predicted by acquisition and basic allocation models. An alternative model, the growth-differentiation-balance-hypothesis, predicts a peak in investment in defense at intermediate resource availability. I estimated the costs of six fitness and/or defensive traits by exposing tadpoles to a resource availability gradient and measured the investment in defense by assessing the plasticity to predator exposure. The results of the experiment show that the costs of responding to predators in defensive traits are not primarily energetic and the investment in defense did not follow the predictions of the models. Costs of defenses caused by increased tail depth and reduced activity were compensated by a reduced developmental rate and a reduced survival. At low resource availability the response costs were not compensated by other fitness traits and the consequence was a reduced survival, while at high resource availability the compensation shifted towards an increasingly reduced developmental rate.

Introduction

Most organisms experience high predation rates and have evolved multiple defensive traits as a result (Lima 1998). Many of these defensive traits are induced by predation risk and are expressed at the same time (Harvell 1990). The optimal expression of each of the multiple defensive traits is expected to vary between different environments. The expression of each trait should be determined by the trade-off between the effectiveness of defense and fitness costs of expressing the trait (Stearns 1992). The expression of the defensive responses are thought to be costly (Harvell 1990). The costs of defensive responses have to be compensated by fitness traits. In this study defensive traits are traits that affect mortality due to predation (e.g. an increased spine length in *Daphnia*), whereas fitness traits are traits that affect other fitness components (e.g. reproduction) (Lampert et al. 1994). High plasticity in defensive traits cause high costs of responses, which have to be compensated by fitness traits. Plasticity in defensive traits should be positively correlated to plasticity in fitness traits.

For traits that can clearly be assigned to either defense or fitness the predictions are clear: the expression of defensive traits causes costs and fitness traits compensate for these costs. For traits that express fitness and defense at the same time, like body size, predictions are less clear. These traits that express fitness and defense at the same time can either add to the net defense or compensate for cost of responses generated by other defensive traits.

If defensive traits differ in their costliness increased resource availability should result in shifts in trait expression (Fig. 1). Increasing amounts of resources should be allocated to costly and potentially more effective defensive traits when the resource availability is high. Costly traits should increase more in their trait expression with increasing resource availability than lower-cost traits (Fig. 1). Assuming that traits differ in their costliness, the shift in trait expression in response to resource availability should also result in variation of predator-induced plasticity in these traits (Fig. 1). The variation in plasticity in predator-induced responses in defensive traits should then reflect on the plasticity of the fitness traits that compensate for the costs of responses caused by the defensive traits.

The goal of this study was to test three different models developed for trade-offs between costly plasticity, net defense and overall fitness. Two models are allocation trade-off models. The allocation of available resources in one trait is traded off against allocation in another trait (Herms and Mattson 1992). The third model is an acquisition trade-off model. A maximized resource acquisition is trading off against minimized predation (Werner and Anholt 1993,

Abrams 2000). The first allocation model (simple allocation trade-off model) predicts an increased investment in defense with increasing resource availability (Fig. 1). At low resource availability resources are (only) allocated in fitness traits (e.g. maintenance), whereas under high resource availability allocation in both fitness and defensive traits can be afforded. With increasing resource availability investment in defensive traits should increase stronger than investment in fitness traits. An increased plasticity in predator-induced responses is expected at high resource availability (Harvell 1990, Tuomi et al. 1991). This idea is supported by several studies (Werner and Anholt 1996, Van Buskirk and Yurewicz 1998, Relyea 2004).

The second allocation model, the growth-differentiation-balance hypothesis (GDBH, (Herms and Mattson 1992) or similar models (Tuomi et al. 1991, Gomulkiewicz and Kirkpatrick 1992) predict enhanced investment in defense at intermediate resource availability (Fig. 1). At low resource availability the GDBH follows the logic of the simple allocation model. At high resource availability the optimal strategy is to develop or grow as fast as possible. Fast development allows fleeing predation risk by early metamorphosis, and a high growth rate allows a fast reduction of predation probability by increasing size. Investment in other defensive traits should only be made at intermediate resource availability. Such a pattern has been found in *Daphnia* head length (Barry 1995).

The third model, the acquisition trade-off model has been developed for behavioral traits. This model is based on the idea that activity is positively correlated to the amount of acquired resources but trades off against mortality due to predation. At low resource availability, activity should be high because the rare resources have to be collected. Plasticity in behavioral traits should be dominated by the optimization of the acquisition of resources at low resource availability. With increasing resource availability the response to predation risk in activity should be more pronounced, which predicts an increased predator-induced plasticity in behavioral traits at high resource availability (Werner and Anholt 1993), Fig. 1).

To test the model predictions I used *Rana temporaria* tadpoles and one of their most common predators (the dragonfly larva *Aeshna cyanea*) as a model system. Predation is the major mortality reason for amphibian larvae, only about 5-7% survive the larval stage (Riis 1991). It is not surprising that each tadpole express multiple defensive traits at the same time. Tadpoles show high predator-induced plasticity in behavioral and morphological traits. They can flee predation risk in the aquatic environment by metamorphosing early and by increasing body size. They respond to kairomones (predator-released chemical cues) by a reduced swimming and feeding

activity and an increase in their tail depth. All these trait responses are known to reduce predation rate (Van Buskirk and McCollum 2000a). I conducted an experiment in which I exposed tadpoles to a predator environment (with non-lethal cadged predators) and a no-predator environment and varied resource availability to assess the response to predation risk and resource availability for six traits (swimming and feeding activity, tail depth, body size, time to metamorphosis and survival). Predator-induced plasticity, i.e. the difference between the trait expression in an environment with and without predation risk was used to assess investment in defense.

I expected the behavioral traits (swimming and feeding activity) to respond in accordance with the acquisition trade-off model by increasing plasticity with increasing resource availability (Werner and Anholt 1993). The morphological defensive trait (tail depth) should respond as predicted by the simple allocation model by increasing plasticity with increasing resource availability (Harvell 1990, Tuomi et al. 1991) or respond in accordance with the GDBH and peak in trait plasticity at intermediate resource availability (Herms and Mattson 1992). The amount of plasticity should depend on the costliness of the trait (Fig. 1). There are two predictions for body size and developmental rate, because they express fitness and defense at the same time. If body size and developmental rate compensate for costs of plasticity an increase in plasticity with increasing resource availability towards less defended and less fit individuals would occur. Whereas, if body size and developmental rate add to defense the increase in plasticity would go towards better defended individuals at high resource availability. Larger sized tadpoles and early metamorphosing tadpoles are less vulnerable to predation, and heavy and early metamorphs expresses high fitness (Altwegg and Reyer 2003). One could expect that tadpoles grow fast and metamorphose early when exposed to predation risk. However, tadpoles usually respond to predation exposure by an overall reduced growth rate and prolonged time to metamorphosis, indicating a compensation for other costs of responses (Van Buskirk and McCollum 2000a, b, Benard 2004). Survival, a pure fitness trait that holds no efficiency in defense, is predicted to compensate for the costs of defense responses and therefore increase in plasticity with increasing resource availability.

Methods

This experiment was designed to assess the response to predators on morphological and behavioral defensive and fitness traits along a resource availability gradient. Some of the models are based on predictions about different growth rates. To manipulate growth rates independent of

resource availability, the experiment was carried out in two different environments, i.e. a cold and a warm environment. For the experiment I used *R. temporaria* tadpoles from clutches of a population at 1159 m elevation near Wildhaus, Switzerland. I reared the tadpoles in 0.28 m^2 outdoor artificial ponds filled with 80 liters at the University of Zürich. At the onset on 4 May 2002, the ponds were stocked with tadpoles which were 4-5 days old (15 mg, stage 24-25, Gosner 1960). Every pond received 20 tadpoles (71 tadpoles/m^2) consisting of two individuals each from ten clutches. The experiment had two temperature treatments (warm and cold), two predator treatments (non-lethal predator and no-predator) and five food levels (resource availability levels), replicated in five complete randomized blocks (100 ponds total). The tadpoles were moved to new ponds when the quality of the water in any of the ponds degraded, this was done on May 22, June 4 and June 18. The experiment was terminated on June 26.

Treatments

For manipulating temperature the warm treatment was equipped with an aquarium heater set to a temperature of 19 C°. Dataloggers in 8 ponds confirmed that the average temperature difference between treatments was 1.64 ± 0.047 C°. The aquarium heaters heated the water for 16h during the day, while during the night the heater was switched off. This was done to ensure similar daily temperature fluctuations in both treatments. In addition, the outdoor field where the ponds were set up was covered with 50% shade cloth to lower the overall temperature and therefore increase the difference between the cold and warm temperature treatment.

The five resource availability levels were 1%, 2%, 4%, 8% and 16% of tadpole body mass fed per day. The amount of food was calculated by weighing two extra sets of twenty tadpoles for each resource availability level just before each feeding. After May 21 the average weight of the experimental tadpoles in each treatment was measured weekly and the amount of food was based on these averages. The tadpoles were fed twice a week with a mixture of ground rabbit chow and fish food.

For the predator treatment each pond contained one floating cage (~1 L volume), which contained either one final instar dragonfly larva (*Aeshna cyanea*) or was left empty. The dragonfly larvae were fed 300 mg of *R. temporaria* tadpoles three times a week, and the dragonflies within the predator treatment were rotated each time they were fed to equalize possible difference among individual *Aeshna*. In no-predator ponds I switched cages to control for effects of disturbance.

Sampling morphology, behavior, life-history and survival data

I measured morphology by randomly selecting five tadpoles from each pond and photographing them in lateral and ventral view on May 21. The tadpoles were weighed and promptly returned to the pond. I used image analysis software to measure the tail depth and to estimate body size as the centroid size calculated from 21 landmarks positioned in three-dimensional space (Bookstein 1991). The size-corrected tail depth was the residual obtained from regressing the tail depth for all measured tadpoles against body size and the square of body size. Morphology and weight could not be measured in two, respectively one pond, due to a technical problem.

I recorded behavior data by instantaneous sampling the activity of the visible tadpoles as swimming, feeding or resting (Horat and Semlitsch 1994). The data were recorded by visiting each pond four times over a three and a half hour period on May 25, one day after the dragonflies had been fed.

Given that all tadpoles entered the experiment at the same age and date, I used date of metamorphosis as a measure of developmental rate. Tadpoles that reached forelimb emergence (Gosner stage 42) were removed from the ponds and kept in individual boxes with little water until the tail was absorbed (stage 46). The date when metamorphosis was completed was noted. The ponds and boxes were checked for metamorphs at least every second day.

Those tadpoles that had not emerged by the termination of the experiment (June 26) were noted as metamorphosed on June 27. All tadpoles that accomplished the four emerged limbs stage (stage 42, Gosner 1960), by June 26 were noted as surviving tadpoles. Tadpoles dying before June 26 or not accomplishing the four emerged limbs stage by June 26 were considered to be non-survivors. The method I used to measure developmental rate underestimates the amount of variance in age at emergence, which is a conservative way of analysis, and it affects the amount of variance in survival most likely by overestimating it. The alternative would be to exclude and therefore discard all the information on tadpoles which have not metamorphosed by the termination of the experiment. This would increase the variance in age at emergence and most likely reduces variance in survival.

Statistical analyses

Predator-induced plasticity was the absolute difference between the non-predator and predator treatment for the different traits. I used absolute values to have a direct scale unit with the exception of tail depth, which was corrected for body size. None of the trait response curves

was fundamentally altered nor would the interpretation of the results change if I analyzed relative values. Two tests were performed for each trait. First, I tested the overall effects of predators, temperature and food and possible interactions on behavior, morphology and life-history traits with a mixed model including block as random factor, predator and temperature as categorical fixed factors, and the logarithm of food levels as covariate. Each pond was defined as independent unit. For all traits I used the pond means in the analysis. Survival and behavioral responses were recorded as ratios and therefore were arcsin-square-root transformed before analysis.

I performed a second set of tests to describe the shape of the predator-induced plasticity along the resource availability gradient. Here, I used model selection based on Akaike's Information Criterion of small samples (AICc) on three candidate models describing the resource availability effect on the plasticity for the different traits. The support for each model was assessed for each trait separately. The three candidate models included a) a intercept only model, which describes no change in plasticity along the resource availability gradient (no-cost control model), b) a linear term for the resource availability effect (acquisition trade-off model and simple allocation model), and c) a linear and quadratic term for the resource availability treatment (GDBH). I calculated the Akaike weight and evidence ratio to determine how much better the best fitting model was supported in comparison to the other models (Burnham and Anderson 2002). I used model selection and not a second-order model, because I was interested in the overall support for the model and did not want to test the significance of each single parameter in the models.

Results

Defensive traits

Tail depth of *R. temporaria* tadpoles responded most to the predator-exposure (Fig. 2a, $F_{1,86}$= 33.93, p<0.0001), with predator-exposed tadpoles having 11.6 % deeper tails than predator-naïve tadpoles. The plasticity in tail depth between predator-exposed and predator-naïve tadpoles did not change with increasing resource availability and was therefore best explained by the no-cost model with only an intercept (Fig. 2g, Table 1).

Both behavioral traits responded strongly to predators (feeding $F_{1,88}$= 13.9, p<0.0001; swimming $F_{1,88}$= 74.5, p<0.0001), but only swimming activity responded to resource availability (Fig.2b & c, $F_{1,88}$= 9.58, p<0.0026). Feeding activity decreased in response to predator-exposure

by 34%. The swimming activity decreased in response to predators by 72%, and with increasing resource availability it increased by 39%. The plasticity in feeding activity between predator-exposed and predator-naïve tadpoles was greatest at intermediate food levels and was therefore best explained by a curvilinear relationship (Fig. 2h, Table 1), while the plasticity in swimming activity between predator-exposed and predator-naïve tadpoles did not change with increasing resource availability and was best explained by a intercept only model (Fig. 2i, Table 1).

Traits that combine fitness and defense

Age at metamorphosis was affected by all factors (predator, $F_{1,88}$= 8.58, p=0.0043; temperature $F_{1,88}$= 6.81; p=0.0106; logarithm of food $F_{1,88}$= 511.1, p<0.0001, Fig. 2d), though the temperature effect became non-significant after Bonferroni-correction ($F_{1,88}$= 6.81; p=0.0106). At high resource availability tadpoles metamorphosed about 8 days earlier than at low resource availability and predator-naïve tadpoles metamorphosed about two days earlier than predator-exposed tadpoles. Not only were the treatment effects significant, but the effect of temperature on the day of metamorphosis was also more pronounced at high resource availability than at low resource availability ($F_{1,88}$= 46.67, p<0.0001, Fig. 2d). The effect of predators on age at metamorphosis increased along the resource availability gradient and was best described by a linear relationship (Fig. 2j, Table 1).

Tadpole mass responded strongly to resource availability (Fig. 2e, $F_{1,88}$= 318.3, p<0.0001). At high resource availability tadpoles weighed three times more than at low resource availability. The effect of predators on tadpole mass increased only at the highest resource availability (Fig. 2k) and the plasticity along the resource availability gradient was best explained by a curve-linear relationship (Table 1).

Fitness traits

Survival increased with increasing resource availability and was reduced under predation risk (Fig. 2f, logarithm of food $F_{1,88}$= 121.7, p<0.0001; predation $F_{1,88}$= 11.87, p=0.0009). At high resource availability survival was almost three times higher than at low resource availability, and 11% more (non-lethal) predator-exposed tadpoles died than predator-naïve ones. At low resource availability the survival of predator-naïve tadpoles was higher while at high resource availability the predator-exposed tadpoles survived slightly better (Fig. 2f, $F_{1,88}$= 9.22, p<0.0031). The survival advantage of predator-naïve tadpoles decreased with increasing resource availability (Fig. 2l) and was best explained by a linear relationship (Table 1).

Discussion

Tadpoles exhibited the predicted and previously described responses to resource availability and to predation risk. Costs of defenses caused by increased tail depth and reduced activity, were compensated by a reduced developmental rate and reduced survival. The compensation shifted between fitness traits along the resource availability gradient. At low resource availability the response costs may not be compensated by other traits and the consequence was a reduced survival, while at high resource availability the compensation shifted towards an increasingly reduced developmental rate. Only at the highest resource availability a reduced body mass in predator-exposed tadpoles partly compensated for the response costs.

The defensive traits, swimming and feeding activity and tail depth required little resources, because they did not show strong responses to the resource availability. These traits did not respond as predicted by the acquisition or simple allocation model. However, plasticity agreed with the argument of allocation theory that when trait costs are small individuals can show full plasticity even at low resource availability (Tuomi et al. 1991). The plasticity of these defensive traits did not increase over the whole range of increasing resource availability and there was no (strong) effect of the resource availability on the trait expression. The lack of an increase in plasticity shows that there was no increased investment in defense with increasing resource availability. The lack of a resource availability effect implies that the costs of predator-induced responses in activity and tail depth are not energetic and therefore no resource allocation shift occurs. However, non-energetic costs are required to explain why these cheap plastic traits have not become fixed in the course of evolution (Harvell 1990). The costs of responses also become obvious by the compensation response in fitness traits.

There were some differences in the plasticity in the defensive traits. Feeding activity was the only trait that responded in its plasticity in agreement with the GDBH. There was no support for the acquisition trade-off model. Feeding activity showed the strongest response to predators at intermediate resource availability, which indicates a fine tuning in plasticity to the resource availability. Theory predicts that at low resource availability the optimal feeding activity should be mainly affected by resource availability and not the predation risk, therefore little predator-induced plasticity would be expected, while at high resource availability the system should be more defense driven (high predator-induced plasticity). Feeding activity agreed with theory at low resource availability, but not at high resource availability. The difference in my result and the model predictions in feeding activity could be explained by a lack of a direct link between

resource acquisition and feeding activity which has been questioned recently in tadpoles (Steiner unpublished) and a number of damselfly larvae (McPeek 2004). This could explain why a potentially low cost behavior trait agreed with a model which is developed in the context of resource allocation shifts between traits.

Support for my findings and disagreement with the acquisition trade-off model comes from the few studies that manipulated resource availability. All of them failed to find a significant interaction between food level and predator treatment in behavioral traits (Anholt and Werner 1995, Laurila et al. 1998, Laurila and Kujasalo 1999, Anholt et al. 2000, Peacor 2002). However, many studies which assumed to manipulate resource availability by manipulating densities found an interaction between density and predator effect in the behavior traits as proposed by the acquisition trade-off model (Werner and Anholt 1993, Relyea 2004). Those differences in results between the two groups of studies suggest that competition effects cannot solely be seen as resource manipulation effects.

The traits that express defense and fitness at the same time compensate for the costs of responses in defensive traits. The results show that developmental rate comes out as a fitness trait and not a defensive trait. Body mass tends to be more a fitness trait than a defensive trait, though there is no general (negative) plasticity to predation risk in mass. At low resource availability the low plasticity in developmental rate and the lack of compensation for costs of responses in body mass is traded off against a high plasticity in survival, while at high resource availability the trade off shifts and the low plasticity in survival is traded off against the high plasticity in development rate and body mass. Such a compensation effect of high plasticity in survival at low resource availability has been found in other studies (Peacor 2002). My results propose a shift in allocation of resources from development and growth to survival (maintenance and storage) with increasing resource availability. It shows that compensation by a reduced body mass does not payoff and the optimal growth rate (as defined by the growth rate in the non-predator environment) should be achieved irrespective of the predation risk at least up to the very high resource availability where we see a reduced body mass in predator exposed tadpoles. At high resource availability the best strategy to compensate for costs of responses is a reduced developmental rate. The resource availability limitation has a more severe effect on developmental rate than the predation risk effect. Therefore at high resource availability individuals might be able to compensate for the costs of defenses by an increased time to metamorphosis; however, at low resource availability an additional delay in time to metamorphosis might hold increasing costs, because it is thought

that the costs of delayed metamorphosis increase towards the end of the season (Altwegg and Reyer 2003).

Conclusion

Defensive and fitness traits seem to have coevolved, because they are correlated and are compensationally integrated (DeWitt and Langerhans 2003). All defensive traits showed a strong response towards better defended individuals when exposed to predators, but they did not respond (strongly) to resource availability. The fitness traits compensate for these response costs, but the major burden of compensation is shifted from survival to developmental time with increasing resource availability. The fitness traits that also express defense (developmental time and size) responded in a non-adaptive way in respect to defense, because tadpoles reduced their size and increased developmental time with predator exposure. However, developmental time and size compensate for costs of responses in the defensive traits (activity and tail depth). The compensation is an adaptive response on the individual level but not on a trait specific level.

Not all variations in costs and compensation for these costs are directly linked. The increased activity in feeding at high resource availability should result in an increase in available energy that can be allocated to other traits, though there is no such response in any of the measured traits; conversely there is a reduction in body mass at the highest resource availability. The same lack of a direct link between defensive and fitness traits becomes obvious by the temperature response in time to metamorphosis. At low temperature there is a potentially greater compensation due to a prolonged time to metamorphosis, while this greater compensation is not reflected in any other trait, because none of the other traits responded to temperature variation. The overall pattern of costs caused by defensive traits and compensation in fitness traits could be shown; however, some of the fine tuning and linkage of traits remains to be explained.

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Table 1: Model selection procedure to evaluate support for three candidate models including an intercept only, a linear (intercept + logfood) or non-linear (intercept + logfood + logfood^2) relationship between predator-induced plasticity and resource availability for six different traits. The AICc and Akaike weight of the best supported model is boldfaced. The evidence ratio 1 describes how much better the best fitting model is supported in comparison to the second best model and evidence ratio 2 describes how much better the best fitting model is supported in comparison with the least supported candidate model.

	intercept only		intercept + logfood		intercept + logfood + logfood^2			
	AICc	Akaike weight	AICc	Akaike weight	AICc	Akaike weight	evidence ratio 1	evidence ratio 2
tail depth	**-181.7**	**0.9872**	-177.2	0.0110	-175.4	0.0018	90.0	544.6
feeding	-50.4	0.0259	-50.4	0.0259	**-54.0**	**0.9482**	36.6	36.6
swimming	**-71.3**	**0.9786**	-67.4	0.0198	-64.9	0.0016	49.4	601.8
metamorphose day	198.5	0.0003	**190.5**	**0.9238**	193.0	0.0758	12.2	2980.9
mass	45.3	0.0001	42.8	0.0012	**36.1**	**0.9987**	812.4	9897.1
survival	7.2	0.0003	**-0.8**	**0.8453**	0.9	0.1544	5.5	2980.9

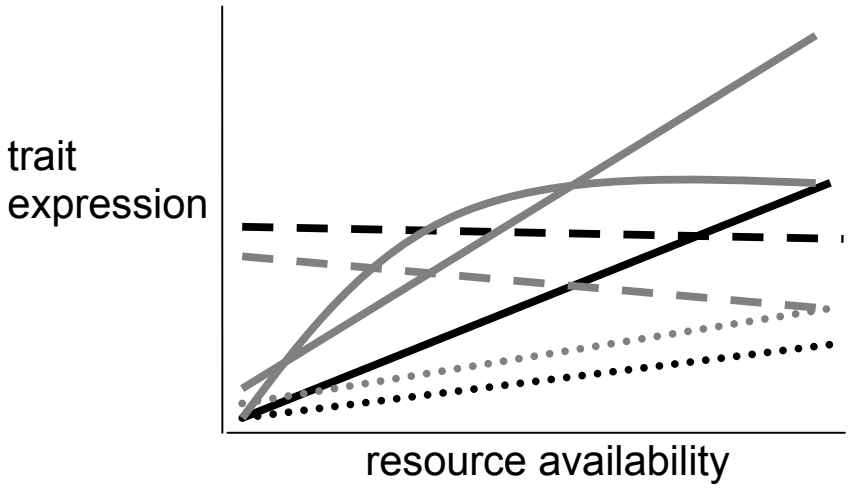

Fig. 1: Expected trait responses to resource availability of costly morphological traits (solid black line), lower-cost morphological traits (dotted black line) and behavioral traits (dashed black line). The grey lines show the expected trait responses of defensive traits along a resource availability gradient under predation risk. The predicted response for the simple allocation trade-off model (dotted and solid linear grey line), the predicted response for the growth-differentiation-balance-hypothesis (solid curved line) and the predicted response for the acquisition trade-off model (dashed grey line). The plasticity is represented by the difference between the black lines and the associated grey line for various defensive trait types.

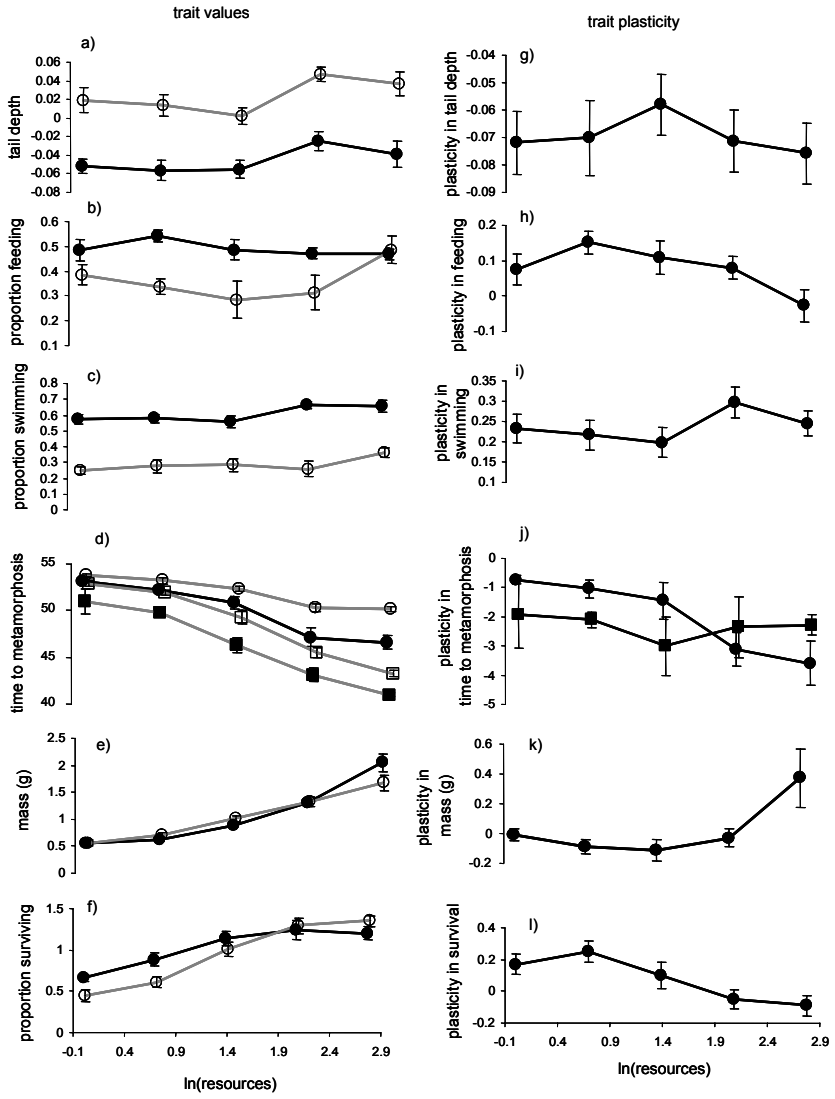

Fig. 2: a-f) Trait expression in six traits of predator-exposed (open symbols) and predator-naïve (filled symbols) *R. temporaria* tadpoles in response to increasing resource availability. For time to metamorphosis (d and j), circles indicate cold temperatures and squares warm temperatures. g-l) Predator-induced plasticity in six traits in response to increasing resource availability. Symbols show means ± SE of five replicates. Plasticity was measured as the difference in the trait expression between non-predator and predator-exposed tadpoles. Feeding and swimming activity were arc-sinus square root transformed, tail depth trait was body size corrected. With the exception of time to metamorphosis, for all traits shown in this figure there was no significant effect of temperature on the response variables. I pooled results from the two temperature treatments in the graphs for simplify the graphical illustration of all the traits except for time to metamorphosis.

CHAPTER 4

INGESTION, DIGESTION AND COSTS OF PREDATOR-INDUCED RESPONSES IN TADPOLES

Ulrich K. Steiner

Abstract

Prey organisms have evolved a variety of defenses to mediate predation risk. Many of these defenses are costly and only expressed under predation risk. Behavioral models assume that the costs of morphological and behavioral defenses stem from reduced feeding and are expressed in reduced growth. Physiological and digestive mechanism should link the induced behavioral and external morphological responses. I tested these model assumptions, by experimentally investigating the effects of exposure to predators on ingestion rate, intestinal morphology, and assimilation rate in tadpoles. Predator-exposed tadpoles showed shorter guts. Despite a reduction in time spent feeding, predator-exposed individuals ingested the same amount of food and assimilated the food at a higher rate, but did not grow faster. Models predicting increased ingestion with increased feeding to maximize growth are misleading. Instead, optimization models that allow intrinsic costs of ingestion and digestion support my results. Costs of avoiding predators, e.g. a reduced feeding rate, can be minimized by modifying ingestion and assimilation rate in response to predators. However complete physiological compensation for the costs of avoiding predators is apparently not possible; and other physiological costs are required to explain why individuals exposed to predation pressure usually grow slowly.

Introduction

Most organisms are exposed to predators during the course of live. A great variety of defense traits have evolved to mediate predation risk including behavioral, life-historical, morphological and physiological traits (Schultz 1988, Lima 1998, Tollrian and Harvell 1999). The expression of most defense traits is costly. To save costs many defense traits are only expressed under predation risk. Such phenotypic plastic responses are called induced defenses (Harvell 1990, Tollrian and Harvell 1999, Pigliucci 2001). The costs of defense arise from tradeoffs involving allocation of time and resources (Stearns 1992). It is argued that costs of inducible defenses are expressed in reduced growth and ultimately reduced fitness. The reduced growth is caused either by shifts in resource allocation for building or maintenance of specific morphological or chemical defense traits (Harvell 1992, Cronin and Hay 1996, McCollum and Van Buskirk 1996, Barry 2000), or by habitat shifts and decreased feeding activity (Gilliam and Fraser 1987, Sih 1987, Harvell 1992, Werner and Anholt 1993, McNamara and Houston 1994, Cronin and Hay 1996, Werner and Anholt 1996, Lima 1998, Barry 2000, Bridges 2002, Van Buskirk 2002a). A reduced feeding activity results in a reduced food uptake, and as a consequence in reduced growth (Belovsky 1978). There is good empirical evidence that prey organisms reduce their (feeding) activity when exposed to predators (Lima and Dill 1990, Werner and Anholt 1993, Relyea and Werner 1999, Eklöv and Halvarsson 2000, Van Buskirk 2000, McPeek et al. 2001, Peacor and Werner 2001, Richardson 2001, Bridges 2002, Van Buskirk 2002b, McPeek 2004). Particularly in systems with sit-and-wait-predators reduced activity of the prey organisms directly reduces the encounter rate with the predator (Werner and Anholt 1993). Therefore, reduced activity is an effective defense mechanism which reduces mortality due to predation (Werner and Anholt 1993). In most of these empirical studies the organisms showed reduced growth rates when exposed to predators. Though it is assumed that reduced growth is caused by reduced feeding, evidence for this assumption is lacking in most predator-prey systems.

The goal of this study is to test if a decreased feeding activity under predation risk is one of the major costs of responding to predators. I investigated if reduced feeding resulted in decreased food intake, and therefore in a reduced growth. The food intake and the growth are linked by the conversion of food into body mass. Individuals that ingest little food might not always show slow growth, because they might compensate for the little amount they ingest by digesting the food more efficiently and therefore convert the ingested food to a higher degree into

body mass (Steinwascher 1979). I investigated factors that are known to affect digestion efficiency, such as gut morphology and assimilation of food. I used an experimental design that allowed separating each step from feeding activity through ingestion, gut morphology, and assimilation of gut contents to growth. A separation in short- and long-term effects of predator exposure allowed separating morphological from behavioral responses.

In this study, I used a predator-prey model system with tadpoles as prey organisms and dragonfly larvae as predators. Tadpoles respond to predator released kairomones (chemical cues released in the water indicating predation risk) with behavioral, morphological and life-historical responses (Werner and Anholt 1993, 1996, Benard 2004). These induced responses include reduced feeding and swimming activity, and reduced growth (Werner and Anholt 1993, Relyea and Werner 1999, Eklöv and Halvarsson 2000, Van Buskirk 2000, Peacor and Werner 2001, Richardson 2001, Van Buskirk 2002b). External morphological, behavioral and life-historical responses have been intensively studied, but internal and physiological responses to predators have been poorly studied (Relyea and Auld 2004). These internal and physiological responses seem likely to connect behavioral and external morphological responses.

Several internal and physiological responses are known to affect the digestion efficiency and the conversion of ingested food into body mass. High quality food can be digested more efficiently (Horiuchi and Koshida 1989, Kupferberg et al. 1994). Longer guts increase the retention time before food is excreted, and as a consequence increase the digestion efficiency (Wassersug 1975, Horiuchi and Koshida 1989). Ingestion and gut morphology can be easily measured (Eklöv and Halvarsson 2000, Relyea and Auld 2004). However, measuring digestion efficiency is not as easily accomplished (Skelly and Golon 2003). I developed a new technique that measures assimilation of gut content, which should be closely linked to digestion efficiency. Assimilation is the amount of ingested food that is evacuated from the gut before it is excreted. Other people refer to it as gut evacuation.

I had two predictions relating to ingestion and digestion efficiency. First, I predicted that predator-exposed tadpoles which spend less time feeding would ingest less food. This is predicted by many feeding models (Sih 1987, Werner and Anholt 1993, McNamara and Houston 1994, Werner and Anholt 1996, Lima 1998). Feeding activity should be independent from morphology and therefore the reduced ingestion should be a short-term effect of predator exposure. Second, tadpoles that ingest little food can (partly) compensate for the little amount they ingest by digesting the food more efficiently (Steinwascher 1979). Therefore, predator-

exposed tadpoles are expected to digest food more efficiently. They should have longer guts, but these longer guts should only be expressed in long-term predator-exposed tadpoles, because a morphological response like an adaptation in gut length would require some time. I also expected predator-exposed tadpoles to have a higher assimilation rate to increase digestion efficiency. If the predator-induced high assimilation rate were a short-term response it would indicate a physiological or behavioral mechanism behind the assimilation rate; if it were a long-term response to predator exposure it would indicate a slow physiological or a morphological response. Both effects, long guts and high assimilation rate increase the digestion efficiency in predator-exposed tadpoles, and thus could compensate (partly) for costs associated with a reduced time spent feeding.

In the end, the impact of costs of avoiding predators should be buffered, by altering the morphology of the intestines, and assimilating food at a higher rate. For tadpoles, complete physiological compensation for costs of avoiding predators is apparently not possible, because individuals exposed to predation risk usually grow slowly.

Methods

Experimental design

For the experiment I used tadpoles of the pool frog *Rana lessonae* (Camerano, 1882). Their responses to predators are well known and do not vary from responses of many other tadpoles (Van Buskirk 2002a). First, I induced a predator morph (induced tadpoles) by conditioning half of the tadpoles in outdoor artificial ponds with caged predators, the other half was reared as control without predators (naïve tadpoles). In a second step, I exposed both morphs to a predator and a no-predator environment in a short-term experiment. This resulted in a two-by-two full factorial experimental design with two long-term conditioning treatments (non-lethal predator and no-predator; hereafter long-term treatment) and two short-term treatments (non-lethal and no-predator; hereafter, short-term treatment). Afterwards, I measured intestinal morphology and calculated ingestion and assimilation rate during the short-term treatment.

Both parts of the experiment were conducted in 0.28-m^2 plastic tubs filled with 80 liters of aged tap water. At the onset of the 24-day long-term treatment the artificial ponds were stocked with zooplankton and 3.1 g of a mixture of rabbit chow and fish food. Additional supplements of 2 and 4 g of rabbit chow were added 18 (16 August 2002) and 22 days (20 August) after the start

of the long–term treatment. Each pond contained one floating cage with or without dragonfly larvae (*Aeshna cyanea,* Müller, 1764) depending on the treatment. The dragonfly larvae were fed 300 mg of *R. lessonae* tadpoles three times a week. All ponds were covered with shade cloth lids to avoid colonization with predators. The ponds were located in an outdoor field at the University of Zürich, Switzerland, and 10 replicates of each treatment were assigned at random to 20 ponds. Each pond was stocked with seven tadpoles (25 tadpoles/m^2) that came from clutches of eggs produced by pairs of *R . lessonae* collected 22 km east of Zürich. When stocking the ponds on 30 July 2002 the tadpoles were at Gosner (1960) stage 28–29 and weighed on average 82 mg. During the long-term treatment some tadpoles died. I replenished tadpoles from one of the original ten replicates to the remaining nine replicates. This was done to obtain nine complete replicates each containing six tadpoles at the end of the long-term treatment and the beginning of the short-term treatment.

The 4 hour short-term treatment was conducted on 22 August 2002, 24 days after the start of the long-term treatment and one day after the dragonflies had been fed. The goal was to measure ingestion, assimilation and intestine morphology in relation to the long-term and short-term treatment by inducing two visible marks at 2 hour intervals in the gut.

Marks were induced by placing the six tadpoles from each pond for five minutes in a 300 mg/l solution of fluorescent dye composed of 122 mg of fluorescent particles (Magruder Color Company JST–300 Series) and 178 mg of ground rabbit chow. The tadpoles ingested the mixture of color particles and food by normal feeding. This method has been used before for inducing gut marks in tadpoles (Eklöv and Halvarsson 2000) and insect larvae (Malmqvist et al. 2001). The feeding behavior and the time the food abided in the gut did not differ for individuals with or without marks when exposure to color particles was only for a short time. The use of color particles enabled me to use different color marks for the two morphs, thereby distinguishing individuals from different long-term treatments.

At the onset of the short-term treatment after inducing the first mark in the gut I divided the six tadpoles out of each long-term pond in two groups. Three of the six tadpoles were put into a pond with the other treatment as during the long-term treatment (predator or no-predator), and the remaining three tadpoles were put in a pond with the same treatment as during the long-term treatment. Therefore, after setting the first mark each of the 18 ponds had six tadpoles, three predator-induced and three predator-naïve tadpoles, and half of the ponds had caged predators an half had no-predators. After 2 hours of normal feeding, I induced a second mark in the gut and

released the tadpoles again into the same pond in which they had been feeding for the previous 2 hours. Another 2 hours later, 4 hours after the start of the short-term treatment, the tadpoles were sacrificed by an overdose of the anaesthetic benzocaine and preserved in 10% formalin for further analysis (Fig. 1).

Measurements, calculations and analysis

Preserved tadpoles were weighed. After that they were dissected and gut width, gut length, the distance from the oesophagus to the second mark and the distance from the second mark to the first mark was measured (Fig. 1). These measurements were used to calculate the volumes of the three different parts of the gut, assuming circular morphology of the gut.

I defined absolute ingestion as the amount of food ingested within the last 2 hours minus the amount of food that was already assimilated (removed from the gut) within these 2 hours. The relative ingestion was defined as the gut volume between the oesophagus and the second gut marker divided by the total gut volume (Fig. 1). I defined assimilation by calculating the proportion between the amount of undigested food that had been ingested 2 to 4 hours before the end of the short-term treatment divided by the amount of undigested food that had been evacuated within the last 4 hours (Fig. 1). For these calculations, I had to assume a constant feeding rate over the 4 hours of the short-term treatment and a direct correlation between volume and amount of food in all parts of the guts. In using intervals of 2 hours the ingestion and assimilation data related directly to ingestion and assimilation rates. Ingestion rate and assimilation rate always refer to a certain proportion or amount ingested or assimilated per time unit, therefore referring to the speed of ingestion or assimilation, while assimilation efficiency refers to the total amount evacuated regardless of the time the food abides in the gut before it is excreted. Two of the 108 individuals were excluded from the analysis, because one or both marks had already been excreted.

Gut morphology measurements and gut volumes were size corrected by taking the residuals of each trait after regression against body mass and body mass squared. With these residuals a repeated measures ANOVA (SAS 8.02 proc glm) with short-term treatment as within-subject effect and long-term treatment as between-subject effect was used. The unit of observation was the mean of three tadpoles that were reared in the same long-term pond and experienced the same environment in the short-term treatment.

Results

The tadpoles in this experiment showed the same suite of responses in respect to behavior (e.g. reduced feeding) and external morphology (e.g. increased tail fin depth) as shown in earlier experiments (Steiner unpublished). However, there were no differences in the final body mass between induced and naïve tadpoles. Body mass did not differ between the short-term treatment, but a strong interaction between the long- and short term treatment occured (Table 1, Fig. 2). Induced tadpoles were 16.5 % heavier when they were in the predator environment during the short-term treatment than the ones in the no-predator environment. Naïve tadpoles altered their mass in the opposite direction in the short-term predator treatment. They were 7.1% lighter in the no-predator environment than in the predator environment.

Morphology of gut

The intestinal morphology was mainly affected by the long-term treatment. Induced tadpoles had guts that were about 6.0 % shorter than those of naïve tadpoles (Fig. 3a and Table 1). This shows that tadpoles adjust their gut length to long-term predator exposure, while in the short-term treatment the time was insufficient for a reaction that affects gut length. However, the long-term effect was more pronounced in the predator environment in the short-term treatment (8.3 % shorter guts) than in tadpoles that were in the no-predator environment during the short-term experiment (3.7 % shorter guts). This shows that there is also a small short-term effect on gut length, which is most likely explained by a trade-off between gut width and gut length. If the gut is heavily filled it gets wide and short but holds more volume, while little filled guts get narrow and long. This idea is supported by my results on gut width. Tadpoles in the predator environment during the short-term treatment showed 4.6 % wider guts than tadpoles in the no-predator environment (Fig. 3b and Table 1). Total gut volume was also mainly influenced by the short-term treatment (Table 1). The short-term effect on gut width and gut volume indicates that tadpoles in predator environments fill their guts more than tadpoles in no-predator environments.

Ingestion and assimilation of gut content

The absolute amount of food ingested within the last 2 hours did not differ between induced and naïve tadpoles (Fig. 4a and Table 1); however, induced tadpoles ingested 19.9 % more food than naïve tadpoles based on relative amounts (Table 1). Tadpoles in the predator environment during the short-term treatment had 11.8% less undigested food in their guts than tadpoles in the no-predator environment during the short-term treatment (Fig. 4b, Table 1). This

result shows a higher assimilation and most likely a more efficient use of ingested food in tadpoles in the predator environment during the short-term treatment.

Discussion

I could show that the common assumption that a high feeding rate results in a high amount of ingested food and therefore in a high growth rate is misleading. Tadpoles can ingest the same amount of food during reduced time they spent feeding. They can also reduce the impact of avoiding predators by altering the morphology of the intestines, and assimilating food at a higher rate. Even though predator-exposed tadpoles ingest the same amount of food and assimilate the food at a higher rate they do not grow faster. These finings support other studies in insect larvae (McPeek 2004). More active damselfly larvae did not ingest more food but were depredated more often. Despite no difference in ingestion or assimilation rate, the species less defended against predation was able to convert food more efficiently into body mass in predator environments (McPeek 2004). This implies that other physiological mechanisms than assimilation affect the conversion of ingested food into body mass under predation risk.

The positive link between feeding activity and ingestion, which is predicted in many behavioral models does not exist in my study. Fast behavioral responses of tadpoles to changing predation risk have been demonstrated (Eklöv and Halvarsson 2000, Van Buskirk 2002a). The tadpoles in my experiment also should adjust their behavior to their present environment. This implies that tadpoles in predator environments reduce their feeding activity. However, the reduction in feeding did not lead to a reduced amount of ingested food in induced tadpoles in the short-term treatment (Table 1, Fig. 4a). Tadpoles in the predator environment can compensate for the reduced time they spent feeding by ingesting the same amount in less time spent feeding. Another mechanism how tadpoles can reduce the impact of avoiding predators is the expression of a high assimilation rate of induced and naïve tadpoles in the predator environment during the short-term treatment (Table 1, Fig. 4b).

My data show that ingestion is affected by a long-term response, suggesting some specialized morphology, e.g. a filter apparatus that allows induced tadpoles to ingest more food in less time. Such a specialized trait however must bear extra costs, because optimal foraging theory suggests that the naïve tadpoles would develop the same morph or habits if there were no extra costs involved (Stearns 1992). In my study, reduced feeding activity in combination with an

increased ingestion rate had no significant effect on growth rate of induced tadpoles, but the two traits might have been traded off against each other (Table 1).

Based on the prediction that morphogenetic changes are unlikely to explain any of the effects of the short-term responses, because morphology would need more time than 4 hours to respond to the new environment, we can explain the difference in ingestion rate (long-term effect) between the two morphs by morphogenetic changes, but not the response in assimilation rate in the short-term experiment. A behavioral cause for a high assimilation rate seems unlikely, because it should be directly linked to the ingestion rate; but the difference between the response in ingestion and assimilation rate to the long- and short-term treatment does not support this idea. Behavior could increase the assimilation rate by more selective feeding for high quality food (Kupferberg et al. 1994). However, more selective feeding would likely require more time spent feeding or would lead to a reduced amount of ingested food (Kupferberg et al. 1994) which is not supported by my data. Therefore behavioral causes to explain the fast response are unlikely. I think a fast responding physiological cause is the most likely explanation for the high assimilation rate of tadpoles in the predator environment. Despite lacking data on any physiological traits apart from assimilation a high metabolic rate could be a response that is adaptive. A high metabolic rate might allow high assimilation rates but might be more a side effect of an increase in the response rate to striking dragonflies and an increase in burst speed, which reduces the predation rate (Fitzpatrick et al. 2003). An increased metabolic rate has been suggested as a response to predator exposure in some other species (Rovero et al. 1999, Stoks 2001, McPeek 2004) and there is ambiguous evidence that tadpoles also respond by increased metabolic rate (Steiner unpublished). A high metabolic rate might be energetically demanding to maintain, which explains why tadpoles in a no-predator environment do not have evolved the same response.

Induced tadpoles ingest more food and therefore should be heavier. Body mass of tadpoles should be highly correlated to the amount of food in the gut, because the total body mass is 50% gut content (Calef 1973). The strong interaction between the short- and long-term treatment in final body mass (Table 1, Fig 3) might be explained by the high ingestion of the induced tadpoles (long-term effect) and the high assimilation rate of the tadpoles in the predator environment in the short-term treatment. If tadpoles are in a predator environment they have a high assimilation rate and potentially metabolize more food, therefore reducing their weight quite fast. While in a no-predator environment they still ingest high amounts of food, but do not

assimilate and metabolize the food as fast, which might result in the high observed total mass. The counter argument is that this interaction between treatments could not be detected in the total gut volume (Table 1).

Under the assumption of optimal foraging theory (Seale and Beckvar 1980, Pyke 1984) and life-history theory (Stearns 1992) hidden costs are required to explain why induced tadpoles do not grow faster despite showing reduced feeding activity, ingesting same amount of food, and assimilating food more efficiently. These hidden costs become obvious, because models that are only based on the idea that feeding activity and ingestion are maximized and positively linked (Belovsky 1978, Werner and Anholt 1993, 1996, Abrams 2003) are not sufficient to explain the observed pattern. However, models based on optimal food intake that maximize growth and fitness by balancing the benefits and costs of ingestion are supported by my results (Illius et al. 2002, Yearsley et al. 2002). In these models the costs of food intake are divided into extrinsic costs associated with the activity of feeding, intrinsic costs of food intake itself, and cost associated with digestion (Yearsley et al. 2002). My experiment showed that there is a complex interplay between behavior, morphology and physiology; this interplay adds and counteracts induced costs in different environments. It shows that the role of physiology, often ignored or underestimated by ecologists can alter the interpretation of experiments and add to the complexity of interactions in costs to induced defenses or more general to plastic responses. Very likely other shifts in physiology as response to predation risk await future discovery and description.

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Table 1: Repeated measures ANOVA on morphological traits and feeding traits with the long-term treatment as between subject effect and the short-term treatment and the interaction between short- and long-term treatment as within subject effect. All tests have 1,16 df. The tests, except final body mass, were performed with body size corrected data.

response	long-term F (p)	short-term F (p)	long-term x short-term F (p)
Final body mass	2.49 (0.134)	1.59 (0.225)	**10.9 (0.004)**
Gut length	**6.87 (0.018)**	1.18 (0.294)	**5.96 (0.027)**
Gut width	1.87 (0.190)	**10.87 (0.004)**	0.01 (0.907)
Total gut volume	0.12 (0.730)	**7.12 (0.017)**	0.72 (0.408)
Volume of food ingested in last 2 hours (absolute amount ingested)	1.88 (0.189)	2.39 (0.142)	0.34 (0.571)
Proportion of food consumed in last 2 hours/ total gut volume (relative amount ingested)	**5.44 (0.033)**	0.40 (0.534)	0.38 (0.547)
Proportion of undigested food	0.09 (0.773)	**12.38 (0.003)**	0.06 (0.812)

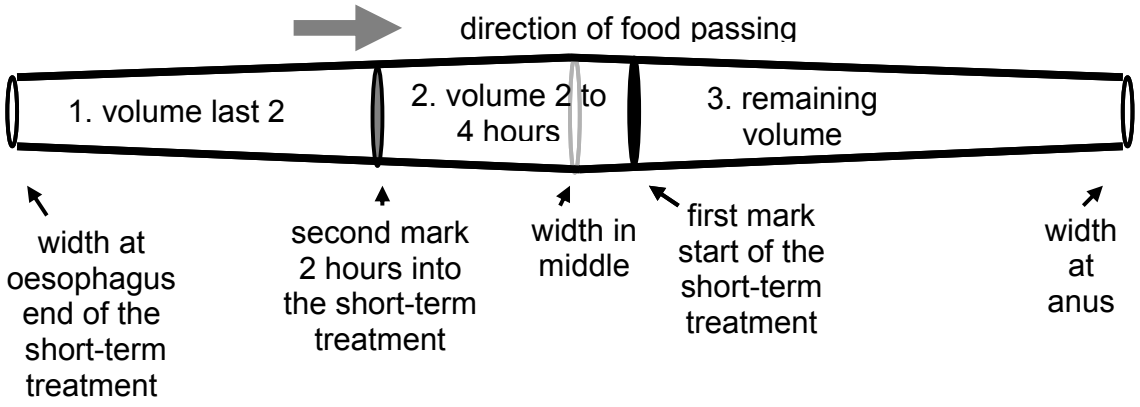

Fig. 1: Gut width measurements were taken at the oesophagus, in the gut middle and at the anus. These measurements were used to calculate the volumes for three gut areas 1. oesophagus to second mark (volume last 2 hours), 2. second to first mark (volume 2 to 4 hours) and 3. first mark to anus respectively (remaining volume). Absolute ingestion is defined by volume1., relative ingestion by volume1./(1.+2.+3.), and assimilation by volume 2./(1.+2.).

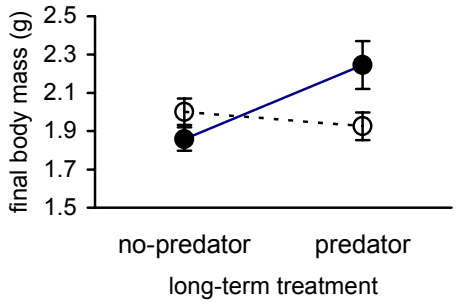

Fig. 2: Final body mass responses of long-term predator-induced and predator-naïve *Rana lessonae* tadpoles in a short-term treatment, with a predator and no-predator environment. Symbols show means ± 1 SE of nine replicates in the short-term environment with filled symbols for the no-predator and open symbols for the predator environment.

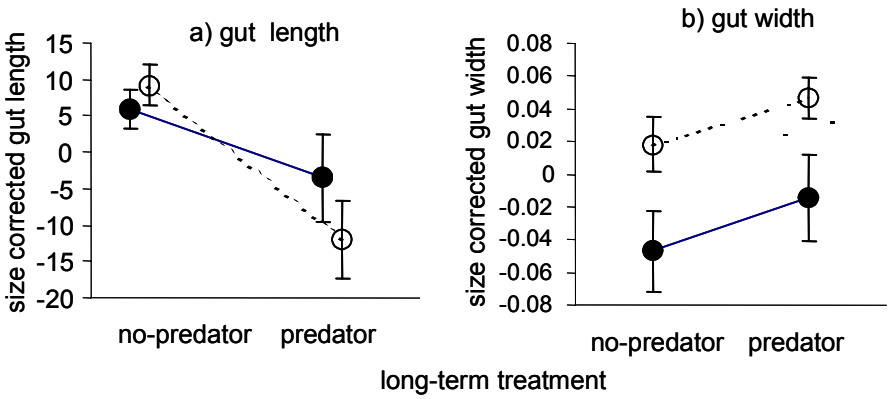

Fig. 3: Body size corrected intestinal morphological responses, a) gut length, and b) gut width of long-term predator-induced and predator-naïve *Rana lessonae* tadpoles in a short-term treatment, with a predator and no-predator environment. Symbols show means ± 1 SE of nine replicates in the short-term environments with filled symbols for the no-predator and open symbols for the predator environment.

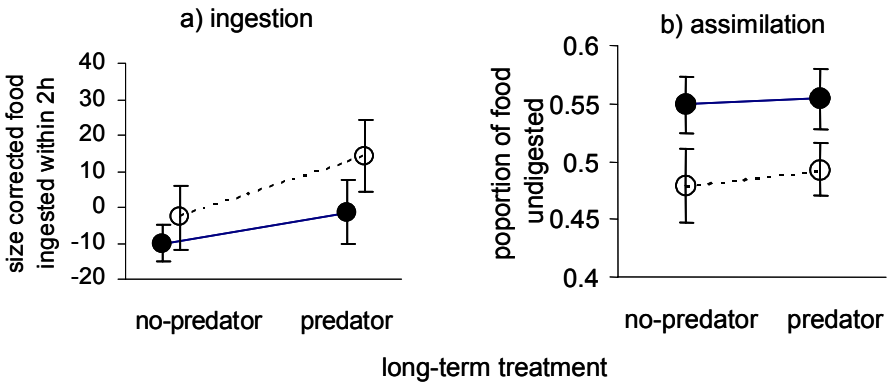

Fig. 4: Ingestion (a) and assimilation (b) responses of long-term predator-exposed and predator-naïve *Rana lessonae* tadpoles in a short-term treatment, with a predator and no-predator environment. Symbols show means ± 1 SE of nine replicates in the short-term environment with filled symbols for the no-predator and open symbols for the predator environment.

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